Radial Patterns of Wood Features Reveal Site-Specific Tree Growth Dynamics in the Congo Basin

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Summary

Tropical forests cover ~7% of the terrestrial surface and stock ~25% of the terrestrial carbon. They play an important role in the biogeochemical cycles and provide short- and long-term services to the society. However, they are increasingly threatened by climate change and anthropogenic pressure. There is a pressing need to better understand the effects of climate change on tree growth in order to predict its impact on forest composition and their ability to provide services.

Until recently, forests responses to climate change have been investigated by experimenting on leaves or by documenting changes on the population level. However, our knowledge of the influence of gradual climate change on tree growth remains limited. The study of tree rings allowed reconstructing past climate and studying its influence on tree growth in the temperate regions. Its application in the tropical context, however, is hampered by the lack of a strong seasonality of growth-limiting factors. There is a great variability in growth-ring distinctness in the tropical species and further research is needed to correctly identify ring boundaries and use them in tree growth studies.

In this thesis, we explore the potential of wood anatomy and wood density variability to reveal trees responses to environmental variations. We assess the variability of tropical tree-rings distinctness and how they are influenced by the environment. The work is subdivided in three chapters, each focusing on a specific question. In particular, we studied (i) the variability of growth-ring distinctness and anatomy in the Tropics, (ii) the relationship between wood anatomy and wood density and the potential of the latter to act as a proxy for studying the variability of anatomical features and (iii) the influence of the seasonality of precipitation patterns on wood density variability.

First, we described the anatomical features involved in the demarcation of tree-ring boundaries and assessed their contribution to tree-ring distinctness of more than 100 species. We found a large intra- and inter-species variability and pointed out that current most widely spread definitions of tree-ring distinctness don’t fully grasp the complexity of wood anatomy in the tropics. Instead of focusing on tree-ring detection, we propose studying the pith-to-bark variability of anatomical features to reconstruct past tree growth and estimate the effects of climate.

Secondly, we automated the measurements of parenchyma and vessels proportions and the ratio lumen/total fibre size and plotted them against the corresponding high-resolution densitometry profile of eight tropical species. We showed that wood density is driven by fibres anatomy and that wood anatomy – wood density relationship is species-specific. Our study suggests that wood density can be used as a proxy to study wood anatomical variability.

Finally, we used pith-to-bark density profiles to compare tree growth patterns of thirteen species along a precipitation gradient. We used wavelet analyses to derive three descriptors quantifying wood growth patterns: regularity, periodicity and amplitude of variations. We showed that precipitation seasonality impacts wood growth regularity and that wood density variations are more abrupt in sites with a more pronounced seasonality. We also showed that evergreen and deciduous species react differently to shifting precipitation patterns.

We strongly believe these results will favour the emergence of promising tools to study past tree growth and the climate – growth relationship. The automation of the measurements of anatomical features and the use of wood density profiles as proxies will help decreasing the processing time and study larger number of trees in order to increase the statistical power of the analyses. Wavelet analysis has the potential to describe more precisely cyclic signals such as wood density profiles which should help deriving new metrics to study and compare different trends of tree growth.

Résumé

Les forêts tropicales couvrent environ 7% de la surface terrestre et stockent environ 25% du carbone terrestre. Elles jouent un rôle important dans les cycles biogéochimiques et fournissent divers services écosystémiques. Cependant, elles sont menacées par l'activité anthropique et le changement climatique. Il y a un besoin pressant de mieux comprendre les effets du changement climatique sur la croissance des arbres afin de mieux estimer son impact sur la composition des forêts et leur capacité à fournir leurs services.

Jusqu'à récemment, les réponses des forêts aux changements climatiques ont été étudiées via des expérimentations en laboratoire ou en rapportant les changements à l'échelle des populations. Cependant, notre connaissance de l'influence des changements climatiques graduels sur la croissance ligneuse reste limitée. L'étude des cernes de croissance a permis la reconstruction du climat passé et l'étude de son influence sur la dynamique de croissance des arbres dans les régions tempérées. Son application dans un contexte tropical reste cependant limitée par l'absence d'une saisonnalité marquée des facteurs limitants la croissance. La distinction des cernes de croissance chez les espèces tropicales est très variable et des études supplémentaires sont nécessaires pour correctement les identifier et les utiliser dans les études dendrochronologiques.

Dans cette thèse, nous explorons le potentiel d'utilisation de l'anatomie et de la densité du bois dans l'étude des réponses des arbres aux changements climatiques. Nous évaluons la variabilité anatomique du cœur à l'écorce et la manière dont le climat l'influence. Le travail est subdivisé en trois chapitres, chacun répondant à une question spécifique. Plus particulièrement, nous avons étudié (i) la variabilité de la distinction et de l'anatomie des cernes de croissance dans les tropiques, (ii) la relation entre l'anatomie et la densité du bois ainsi que son potentiel d'utilisation comme un proxy pour estimer variabilité anatomique et (iii) l'influence de la saisonnalité des précipitations sur la rythmicité de la croissance des arbres via leurs profils de densité du bois.

En premier lieu, nous avons décrit les traits anatomiques impliqués dans la démarcation des limites des cernes de croissance et évalué leur contribution à la distinction des cernes. Nous avons constaté

une grande variabilité intra et interspécifique et mis en avant le fait que les définitions actuelles de la distinction des cernes ne décrivent pas suffisamment la complexité de l'anatomie du bois dans les tropiques. Au lieu de se concentrer sur la détection des cernes, nous proposons d'étudier la variabilité des traits anatomiques depuis le cœur jusqu'à l'écorce dans le but de reconstruire la croissance ligneuse passée et estimer les effets du climat.

Ensuite nous avons automatisé les mesures de proportions de parenchyme et de vaisseaux ainsi que le ratio lumen/diamètre total des fibres dans le plan transversal de la moelle à l'écorce. Nous les avons étudiés en parallèle avec des profils à haute résolution de la densité du bois de huit espèces. Nous avons montré que la densité du bois est influencée par l'anatomie des fibres et que la relation entre l'anatomie et la densité du bois dépend de l'espèce. Notre étude suggère qu'il est possible d'utiliser les profils de la densité du bois comme un proxy pour étudier la variabilité de l'anatomie du bois.

Finalement, nous avons utilisé les profils de densité afin de comparer les patterns de croissance ligneuse de 13 espèces le long d'un gradient de précipitation. Nous avons utilisé l'analyse en ondelettes afin de dériver trois descripteurs de croissance : la régularité, la périodicité et l'amplitude des variations. Nous avons montré que la saisonnalité de précipitations influence la régularité de la croissance ligneuse et que les variations sont plus abruptes dans les sites avec une saisonnalité plus marquée. Nous avons aussi montré que les espèces décidues et sempervirentes ne réagissent pas de la même façon aux variations des patterns de précipitations.

Nous pensons que ces résultats vont favoriser l'émergence d'outils prometteurs pour étudier la croissance des arbres et sa relation avec le climat. L'automatisation des mesures des traits anatomiques et l'utilisation des profils de densité du bois comme proxies vont aider à diminuer le temps de traitement des échantillons et augmenter leur nombre ainsi que la puissance des traitements statistiques. Par la description très fine des signaux cycliques qu'elle permet, l'analyse en ondelettes ouvre de nouvelles perspectives pour la description des profils de variation de la densité du bois et donc l'interprétation de la dynamique de croissance des arbres.

Samenvatting

Tropische bossen bedekken 7% van de landoppervlakte en bevatten 25% van de terrestrische koolstof. Ze spelen een belangrijke rol in de biochemische cyclus en voorzien korte en lange termijn diensten aan de gemeenschap. De huidige toestand van tropische bossen wordt echter bedreigd door klimaatsverandering en antropogene belasting. Er is nood aan het beter begrijpen van de effecten van klimaatsverandering op boomgroei om de impact te kunnen voorspellen op de bossamenstelling en hun mogelijkheid tot het leveren van ecosysteemdiensten.

Voorlopig werden de responses van bossen op klimaatsverandering onderzocht via het uitvoeren van experimenten op bladeren of door het documenteren van veranderingen op het populatieniveau. Onze kennis van de invloed van graduele klimaatsverandering op boomgroei blijft echter beperkt. De studie van groeiringen in bomen laat ons toe om het klimaat te reconstrueren en de invloed hiervan op boomgroei in de gematigde regio's te bestuderen. Het gebruik van deze studie in een tropische context ligt moelijker, vooral door het gebrek aan een sterke seizoenaliteit van groei-limiterende factoren. Er is een grote variabiliteit in de mogelijkheid om groeiringen te onderscheiden in tropische soorten en verder onderzoek is nodig om ringgrenzen correct te identificeren en deze te gebruiken in onderzoek naar boomgroei.

In dit onderzoek verkennen we het potentieel van houtanatomie en houtdensiteit variabiliteit om de respons van bomen op omgevings-variabiliteit in kaart te brengen. We bepalen de variabiliteit van de duidelijkheid van ringgrenzen in tropische bomen, en hoe deze beïnvloed worden door het klimaat. Dit werk is onderverdeeld in drie hoofdstukken met telkens een focus op een specifieke onderzoeksvraag. Meerbepaald, we onderzoeken (i) het gebruik van de definitie van het onderscheid in groeiringgrenzen in de tropen en de daarmee gepaard gaande anatomische karakteristieken, (ii) de relatie tussen houtanatomie en houtdensiteit en het potentieel van houtdensiteit als proxy voor het bestuderen van de variabiliteit in anatomische karakteristieken en tenslotte (iii) de invloed van seizoenaliteit van regenval patronen op de variabiliteit in houtdensiteit.

Eerst worden de anatomische karakteristieken die gebruikt werden om boomringen af te bakenen beschreven en wordt hun aandeel in het onderscheiden van boomringen bepaald voor meer dan 100 soorten. Een grote intra – en inter-species variabiliteit werd vastgesteld waaruit blijkt dat de huidig gebruikte definities om boomringen te onderscheiden niet de volledige complexiteit omvatten van houtanatomie in de tropen. We stellen voor om, in plaats van te focussen op boomring detectie, de merg-tot-schors variabiliteit van anatomische karakteristieken te onderzoeken om zo vroegere boomgroei te reconstrueren en een inschatting te maken van het klimaatseffect.

Vervolgens automatiseren we de metingen van parenchym en vatproporties en de ratio van lumen/wand van de vezels om deze vervolgens te relateren aan het corresponderende hoge-resolutie densitometrisch profiel van acht tropische soorten. We tonen aan dat houtdensiteit bepaald wordt door de anatomie van de vezels en dat de houtanatomie – houtdensiteit relatie soort specifiek is. Onze studie suggereert dat houtdensiteit kan worden gebruikt als proxy om de houtanatomie variabiliteit te onderzoeken.

Finaal worden merg tot schors densiteitsprofielen gebruikt om boomgroei patronen van dertien soorten te vergelijken langs een regenval gradiënt. Wavelet analyse werd gebruikt om drie descriptors te bepalen die houtgroei patronen kwantificeren: regulariteit, prediociteit en de amplitude van de variaties. We tonen aan dat regenval de houtgroei seizoenaliteit beïnvloed en dat variaties meer abrupt zijn in regio's met uitgesproken seizoenaliteit. Daarnaast werd aangetoond dat altijd groene en bladverliezende soorten verschillend reageren op veranderende regenval patronen.

We stellen dat deze resultaten de opkomst van veelbelovende instrumenten om boomgroei in het verleden en de relatie tussen klimaat en groei te onderzoeken zal bevorderen. De automatisering van de metingen van de anatomische karakteristieken en het gebruik van houtdensiteit profielen zal bijdragen tot het verlagen van de verwerkingstijd en zal het mogelijk maken om een groter aantal soorten te onderzoeken met als gevolg een toename van de statistische kracht van de analyses. Wavelet analyse heeft het potentieel om cyclische signalen, zoals houtdensiteitsprofielen, te

beschrijven met een grotere precisie. Deze houtdensiteitsprofielen zouden moeten helpen met het bepalen van nieuwe maatstaven om de verschillende trends in boomgroei te onderzoeken en vergelijken.

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Acronyms

CWT: Continuous Wavelet Transform

DBH: Diameter at Breast Height

DRC: Democratic Republic of Congo

ENSO: El Niño Southern Oscillation

FAO: Food and Agriculture Organisation

GHG: Greenhouse gases

IADF: Intra-Annual Density Fluctuation

IAWA: International Association of Wood Anatomists

INEAC: Institut National pour l'Etude Agronomique du Congo

INERA: Institut National pour l'Etude et la Recherche Agronomiques

IPCC: Intergovernmental panel for climate change

KNN: K-Nearest Neighbour

masl: meters above sea level

RMCA: Royal Museum of Central Africa

UGCT: Ghent University Centre for X-ray Tomography

WD: Wood density

WRB: World Reference Base

WWF: World Wide Fund for Nature

X-ray CT: X-ray Computed Tomography

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Introduction

Climate change: drivers and consequences

The Earth's radiative budget has been unbalanced for at least the last half century (IPCC, 2014) resulting in considerable environmental changes. The Earth's surface warmed at an unprecedented and still increasing rate, passing from 0.65°C to 1.06°C between 1880 and 2012 (IPCC, 2014). Moreover, this increase mainly occurred during the last decades, with average temperatures in the last three decades higher than in all previous ones (IPCC, 2014).

Natural and anthropogenic processes impact the Earth's energy budget which, in turn, alters the climate equilibrium. The main anthropogenic drivers of climate change are the emissions of greenhouse gases (CO_2, CH_4 and the NO_X, with CO_2 contributing to up to 80% of the effects of the GHG), Ozone, Aerosols and the albedo variations due to land surface changes (IPCC, 2014). Today concentrations of CO_2 (408.39 ppm, Dlugokencky & Tans, 2018) are largely out of the natural range (180-300 ppm) recorded in the ice cores of the pre-industrial era.

Fossil fuel burning and deforestation are the most important drivers of CO_2 emissions with respectively 7.8 PgC (GEA, 2006) and 1.1 PgC (Pan *et al.*, 2011) emitted per year. Part of these emissions (55%) is absorbed and stored in the ocean and vegetation reservoirs. The remaining 45% reside in the atmosphere and contribute to the climate change.

These processes are expected to influence the Earth's climate for a long period. Surface temperatures, the frequency and the duration of heat waves and the frequency and magnitude of extreme climate events are expected to keep increasing during the XXI century. Oceans will continue to heat and acidify, while the mean sea level is expected to rise (IPCC, 2014).

All these changes are expected to threaten the ecosystems as we know them and their services impacting the lives of millions of people around the globe. Considerable efforts are needed to address climate change. Different approaches, ranging from the reduction of carbon emissions to the absorption of the emitted carbon, can be adopted. Important quantities of carbon are emitted and absorbed through natural processes and any disturbance of these processes might severely impact

climatic equilibrium. The vegetation is responsible for the largest fluxes of carbon and act as a major carbon sink, making thus the forests important actors in the global carbon cycle and in the mitigation of the climate change (Lewis, 2006; Richardson *et al.*, 2013).

Forests and climate change

Forests cover ~43.5 million km² worldwide (~30% of the land surface; Bastin *et al.*, 2017). They store ~45% of the terrestrial carbon (Sabine *et al.*, 2004) and can sequester large amounts annually: ~30% of carbon emissions due to fossil fuels and land cover change since the 1990s were absorbed by forests worldwide (Denman *et al.*, 2007). Additionally, forests exchange energy, water and influence atmospheric composition (Bonan, 2008). Tree evapotranspiration contributes to the hydrological cycle and the resulting clouds and precipitation help cooling the climate (Bonan, 2008). Finally, they provide short-term (wood, shade, food) and long-term (habitat for endangered species, soil stabilisation, water quality restoration, buffer to extreme climatic events) services to the society (Bonan, 2008; Pan *et al.*, 2011; FAO, 2016).

However, forests are increasingly threatened by climate change and anthropogenic deforestation. Although in the temperate regions the forest area has increased thanks to agricultural areas conversion (+3 million ha), forest area has decreased substantially in the Tropics in the last 15 years (- 7 million ha) leading to a total loss of forest area worldwide (FAO, 2016).

Carbon emissions from deforestation and forest degradation account for approximately 12% of global anthropogenic CO_2 emissions (Le Quéré *et al.*, 2017). Additionally to the increase of the atmospheric CO_2 level, land cover change has biogeophysical impacts on climate via changes in evapotranspiration, surface roughness and albedo (Sitch *et al.*, 2005). As a consequence, the amount of reflected energy and the proportion of energy released as heat and non-heating fluxes (through evapotranspiration) are affected (Turner *et al.*, 2007; Vargo *et al.*, 2013).

The resulting climate change and extreme climatic events can further affect forests, providing positive feedbacks to CO_2 release and global warming (Reichstein *et al.*, 2013). Events such as windstorms,

droughts or wildfires can severely damage forests and hamper their ability to provide services (Moreau, 2016). For example, the past decade, four major storms fell 410 million m^3 of wood, severe droughts in Amazonia in 2005 and 2010 caused the loss of a decade's worth of carbon sequestration and forest fires burned 23000 km^2 in Russia in 2010 (Moreau, 2016).

In addition to the extreme climatic events, more gradual climate changes can also negatively affect forests. Increased temperatures may lead to severe pest outbreaks, such as the bark beetle pandemic that has increased tree mortality and caused forest loss over 130 000 km^2 in Canada since 2004 (Bellassen & Luyssaert, 2014). Leaf phenology, a key process influencing the intra- and inter-annual variability of albedo, water, energy and carbon fluxes, can be affected by temperature and precipitation variations (Morisette *et al.*, 2009; Richardson et al., 2013). Furthermore, several authors predict increased heat-induced tree mortality and vegetation shifts possibly impacting functional composition of the forests (Allen *et al.*, 2010; Ruiz-Benito *et al.*, 2017).

Tropical forests

Tropical forests and woody savannahs cover 12-15% of the land surface (Figure 0-1; FAO, 2006), store about 60% of total global forest biomass (Pan *et al.*, 2011) and are highly productive systems (Taylor *et al.*, 2017). They play a significant role in the water, carbon and nutrient cycles (Bonan, 2008; Spracklen *et al.*, 2012). Due do their disproportionate importance in global cycles, even subtle changes in tropical forests may have important global impacts (Lewis, 2006). Moreover, they host between one-half and two-thirds of the world's species (Malhi & Grace, 2000; Groombridge & Jenkins, 2003) and a change of their habitats may drive many species to extinction (Lewis, 2006). Finally, they provide food, building materials, medicines and other services for populations living in the Tropics (Lawrence *et al.*, 2005; Reid *et al.*, 2005).

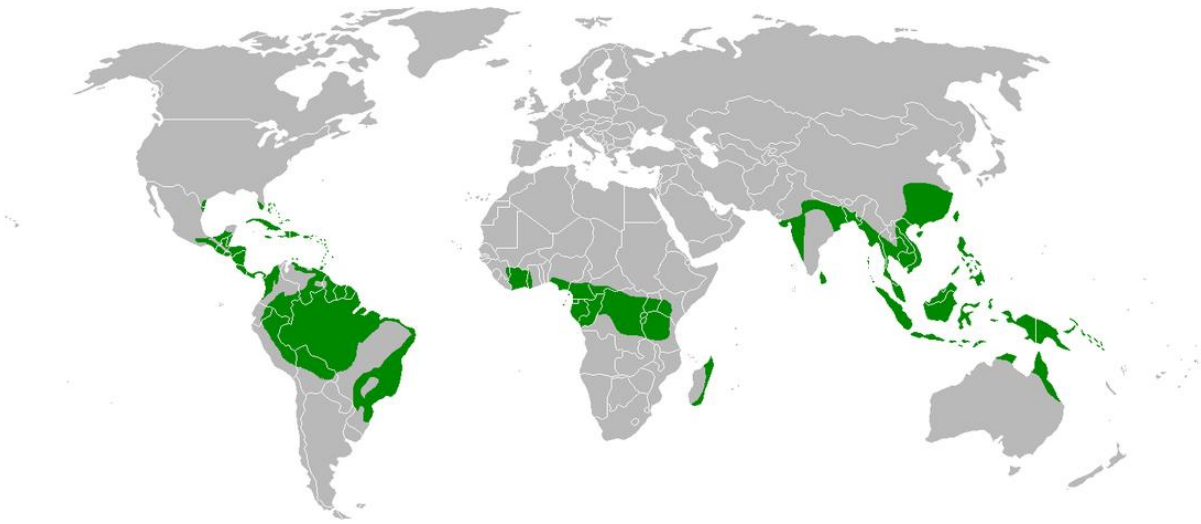

Figure 0-1: Tropical forest distribution around the world.

Tropical forests include a vast array of biomes, ranging from rainforests to wooded savannah systems and comprising mangroves, montane or dry forests (Lewis, 2006). They occur on various soil types (Malhi *et al.*, 2004) and in different environmental conditions with annual precipitation ranging from <500 to >4000 mm and the average duration of stress periods ranging from to 2 to >30 weeks (Fichtler & Worbes, 2012).

Unlike in the temperate zones, seasonal cycles of temperature and photoperiod are weak in the tropics, given the little variation year-round. In temperate zones, winter is the dormant period after which tree growth resumes and continues until the next autumn, forming a growth ring (Mariaux, 2016). In tropical zones, tree growth is influenced by water availability. Favourable and unfavourable growing conditions are driven by precipitation which defines the rainy and the dry seasons. Moreover, the seasonality of precipitation is variable across the Tropics (Fichtler & Worbes, 2012) and its effects may be buffered by the soil water reserve.

Despite the evidence that undisturbed forests act currently as global carbon sinks (Aragão *et al.*, 2014; Fisher *et al.*, 2013; Valentini *et al.*, 2014; Le Quéré *et al.*, 2017), their future effect on climate is uncertain as their resilience to droughts, fires, increasing temperatures, decreasing precipitation and rising levels of CO_2 stays indeterminate (Lewis, 2006; Bonan *et al.*, 2008; Fisher *et al.*, 2013).

There is a growing number of records of changing biomass and fluctuating tree growth rates in tropical forests (Chave *et al.*, 2008; Lewis *et al.*, 2009; Dong *et al.*, 2012). Recent studies have shown that the carbon cycle in the tropical forests is highly responsive to temperature and rainfall variations (Taylor *et al.*, 2017). Brienen *et al.* (2015) have shown a 30% decline in carbon accumulation rates potentially due to the increased average temperature in the Amazon basin and severe droughts have been linked to large carbon losses from Amazonian forests (Malhi *et al.*, 2008; Lewis *et al.*, 2011; Cleveland *et al.*, 2015). In addition, species have varying sensitivities to environmental fluctuations and may respond differently to climate change (Brienen et al., 2016) which may affect competition and the relative abundance of species in the forest (Korner, 2009) and influence carbon fluxes (Zuidema *et al.*, 2013). Laurance *et al.* (2004) showed changes in tree species composition, with disturbance-adapted taxa likely to benefit from the ongoing changes at the expense of the more vulnerable to extinction, disturbance-averse species (Lewis, 2006).

In the future, tropical forests are expected to face increased temperatures and aggravated drought (James *et al.*, 2013, IPCC 2013), and the underlying processes of the aforementioned phenomena are still to be understood. A better understanding of these mechanisms is therefore essential to predict the future of forests (Scheffer *et al.*, 2001; Malhi & Wright, 2004; Phillips *et al.*, 2010, Zuidema *et al.*, 2013).

Tropical forest studies: progresses and limitations

Zuidema *et al.* (2013) compiled previous research efforts of climate influence on forest dynamics and identified four different levels of studies: leaves, individual trees, populations composed of conspecific trees and, finally, communities that correspond to entire forest stands. The most commonly suggested causes for the changing dynamics are shifts in rainfall, temperature, CO_2 concentration and nutrient deposition (Lewis *et al.*, 2009). A range of these studies was performed in permanent sample plots in relatively undisturbed tropical forests. While they provide valuable information on changes in tropical forest dynamics, they present a number of shortcomings such as not allowing for a separation of the

effects of different global change factors (Clark, 2007) or a coarse temporal resolution of measurements (approximately every five years; Zuidema *et al.*, 2013).

The scientific community has a good understanding of the leaf-level physiological responses to changing environmental conditions (Huang *et al.*, 2007; Lloyd & Farquhar, 2008). Leaf photosynthesis responses to variations in CO_2 levels, temperature, precipitation and/or nutrient availability are now well documented (Zuidema *et al.*, 2013).

By contrast, the tree-level is understudied and our understanding of the influence of the environmental changes on tree growth is limited (Zuidema *et al.*, 2013). While drought simulations have been performed on mature trees (Brando *et al.*, 2008), the effects of CO_2 or temperature increase have only been tested on seedlings (Korner, 2009; Way & Oren, 2010). While this information is valuable, it needs to be used carefully as mature and bigger trees responses are likely to differ from seedlings and are also more relevant in carbon dynamics studies (Zuidema *et al.*, 2013; Bastin *et al.*, 2015a).

Tropical tree populations and communities have been monitored for several decades using forest inventories (Lewis *et al.*, 2004; Brienen *et al.*, 2015) or remote sensing (Moreau, 2016). Important shifts in composition (Laurance *et al.*, 2004) and carbon dynamics (Brienen *et al.*, 2015) have been observed in the past years but it remains unclear how climatic changes drive these phenomena (Zuidema *et al.*, 2013). Although the effects of varying precipitation, increasing temperatures or CO_2 levels on forest stands are investigated by drought experiments (Brando *et al.*, 2008; Phillips *et al.*, 2009), eddy covariance observations or the free-air carbon enrichment experiments (Sanchez-Carillo *et al.*, 2015), the results remain sparse in the Tropics and the understanding of the interactive effects of CO_2, temperature and precipitation is limited (Zuidema *et al.*, 2013).

Since the well-described shifts in leaf photosynthesis induced by climate change translate into changes in tree growth at the individual level (Anderegg *et al.*, 2012), the effects of climate change should be studied at the level of trees and aggregated to population and community levels afterwards (Clark et

al., 2011). Furthermore, Zuidema *et al.* (2013) advocate in favour of increasing the time scales of the studies to match the lifespans of tropical trees (Rozendaal & Zuidema, 2011), their acclimation to climate changes (Corlett, 2011) and the temporal scale of interest regarding climate change.

One currently investigated approach that would allow reducing the temporal scale gap is dendrochronology and, more generally, wood anatomy. Variation of the different cell types dimensions and proportions is the result of the competition between their functions under environmental and phylogenetic constraints (Chave et al., 2009). Wood anatomy is a combined result of intrinsic and environmental factors and its study can reveal ecological information and the adaptation to past and present environmental conditions of the studied trees (Wheeler & Baas, 1993). Therefore, wood anatomy and dendrochronology coupled to the long-term plot studies are of special interest for investigating tree response to climate change at the relevant temporal scales (Clark, 2007).

Wood anatomy and tree-ring studies

Wood is a complex tissue and is mainly composed of three cell types assuming different interrelated functions: vessels for water transport, fibres for mechanical strength and parenchyma for nutrient storage and transport (Schweingruber, 1996). Cells are formed, elongated and differentiated in the vascular cambium. All these processes are genetically constrained and driven by different phytohormones (Sorce *et al.*, 2013). However, these processes are also influenced by the environmental conditions, imprinting thus the environmental information in the wood.

Trees periodically face unfavourable growth conditions and respond with cambial dormancy which interrupts or slows down xylem radial growth (Worbes & Fichtler, 2010). By the end of the growing season, photosynthetic activity decreases, resulting in more-or-less important leaf turn-over and cambial dormancy (Singh *et al.*, 2017). The return of the favourable growth conditions drives buds break (Korner & Basler, 2010) and the resume of the photosynthesis and the cambial activity. The early wood differs from the late wood in terms of in the size and shape of the cells, forming thus a visible growth ring marking the end of each growth cycle (Speer, 2010). While in temperate zones,

temperature and photoperiod are the most common factors that trigger cambial dormancy (Fritts, 1976), water availability is the most common growth-limiting factor in the Tropics (Worbes, 1995). Periodic droughts cause seasonal water stress which may result in deciduousness or brevi-deciduousness and reduced cambial activity (Borchert, 1994). During floods, anoxic conditions of the roots also cause leaf fall, cambial dormancy and the formation of a tree ring (Schöngart *et al.* 2002).

Tree rings can be used in various ways to obtain information on tree and forest dynamics (Brienen & Zuidema, 2006), tree ages (Worbes & Junk, 1999), climate reconstructions (Schongart *et al.*, 2004) or changes in the carbon stocks of the above-ground biomass (Schöngart *et al.*, 2010). Their width is proportional to the amount of wood formed during the growth cycle, tree-ring series contain thus information on tree growth and its environment on different time scales (Peters *et al.*, 2015). Yearly variations are often driven by inter-annual fluctuations in environmental variables (*e.g.* temperature and precipitation; Subedi & Sharma, 2013), decadal variations may respond to canopy dynamics (Baker & Bunyavejchewin, 2006), while long-term variations (several decades to centuries) may be driven by gradual environmental changes or ontogenetic trajectories of trees (Briffa & Melvin, 2011; Peters *et al.*, 2015).

Tree rings are linked to leaf photosynthetic activity (i.e. leaf shedding, stomatal regulation). Closely related to precipitation patterns in tropical conditions, leaf shedding is the link between the climatic signal and cambial activity (Borchert, 1999). The leafless period of several neotropical species during the dry season causes lower trunk diameter increments (Lisi et al., 2008; O'Brien et al., 2008). However, there is a high variability in drought tolerances and responses (Worbes *et al.*, 2013) and leaf and cambial phenology get progressively uncoupled from climatic seasonal variations in brevi-deciduous and/or evergreen species (Borchert, 1999). These species, confined to soils with adequate water availability experience moderate water stress and their cambial activity might continue well into the dry season with little to no growth-ring formation.

Tree-ring studies in tropical forests without strong growth-limiting environmental factors can present specific challenges (Groenendijk *et al.*, 2014). Under persistently wet conditions the cambial activity variation can be weak leading to reduced variation in wood anatomical features and decreasing tree-ring boundary distinctness (Moya & Filho, 2009), making tropical tree-ring studies particularly challenging. To efficiently use tree-ring patterns in ecological studies, all ring boundaries need to be determined in a sample (Worbes & Fichtler, 2010). Tree rings can have highly diverse anatomical features but can be reduced to four basic tree-ring types: a) fibres with shortened radial diameter and thickened cell walls, b) marginal bands of axial parenchyma cells, c) periodically recurring parenchyma and fibre bands of different widths and d) varying frequency and diameter of vessels (Worbes, 1985). These features often occur in various combinations and with varying degrees of abruptness and it is essential to consider the general pattern of a sample rather than looking for a specific character delineating a ring (Worbes & Fichtler, 2010).

While clearly defined rings are less common in the Tropics than in temperate zones (Figure 0-2) due to a less pronounced seasonality (Rozendaal *et al.*, 2011), a growing number of tropical species is known to form distinct tree rings (Brienen *et al.*, 2016). A broad variability of ring-boundary distinctness in tropical trees exists: ring boundaries may be distinct throughout the entire stem or only part of it, they can be wedging or interrupted or simply indistinct (Worbes & Fichtler, 2010). The whole concept of growth-ring distinctness is subjective and relies on the operator's judgment and experience, especially for boundaries with gradual anatomical changes (see Fahn, 1962). A training based on temperate or tropical species gives different habits and expectations about growth-ring distinctness which might lead to different labelling of species with intermediate growth-ring distinctness. There is thus a necessity of a clear and broad definition of growth-ring distinctness in order to construct databases suitable for different studies in different environments (Tarelkin *et al.*, 2016).

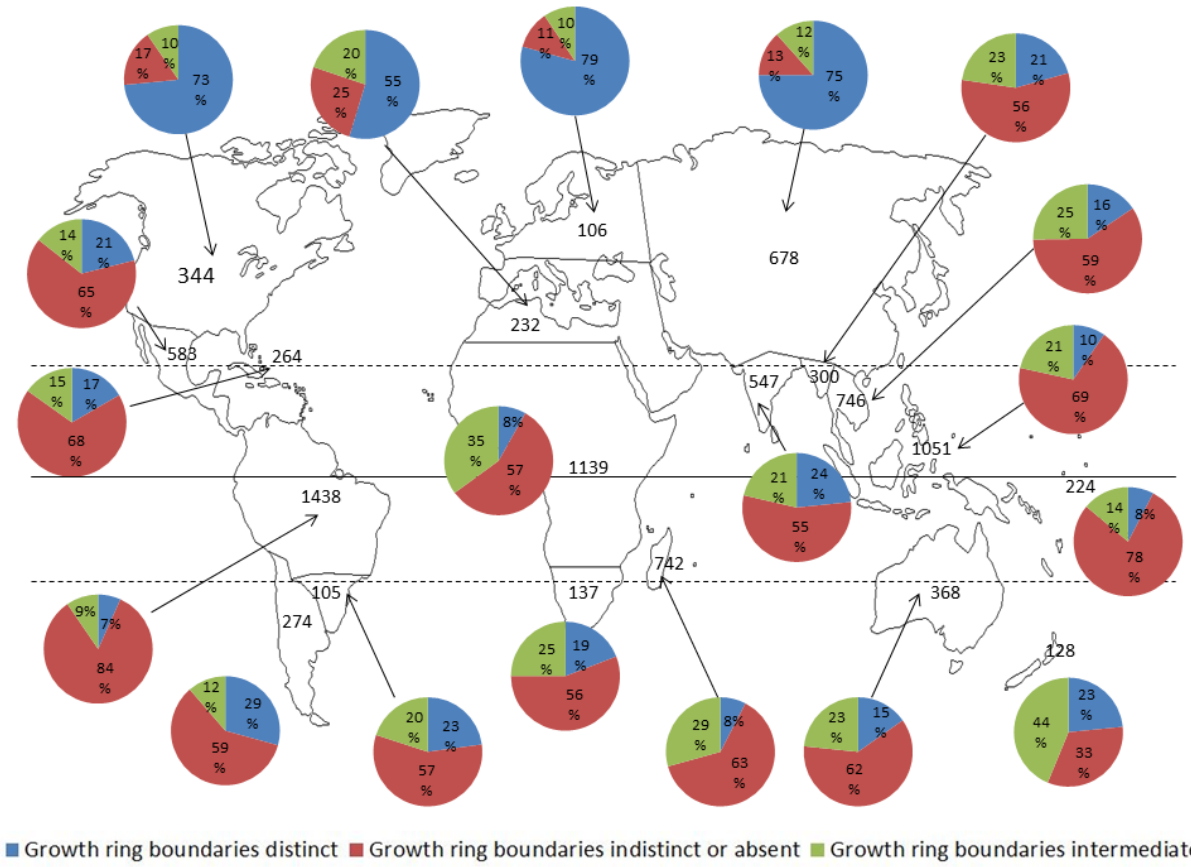

Figure 0-2 : Distribution of tree-ring distinctness as defined by Inside Wood across different biomes (Wheeler, 2011). The full line represents the equator and the dashed lines represent the tropics of Cancer (North) and of Capricorn (South). Species described with distinct ring boundaries (Feature 1 in Inside Wood) are in blue, species with indistinct/absent rings (Feature 2) in red and species with rings described as distinct by some researchers and indistinct by others (both Features 1 and 2) in green. The number in each delimited IAWA region is the amount of species described in the indicated zone.

The annual nature of tree rings is also essential in dendrochronology (Groenendijk *et al.*, 2014). Tree-ring formation depends on the occurrence of triggering environmental factors and can be strictly annual, bi-annual (Gourlay, 1995) or irregular (false rings occurring once-in-a-while; Wils *et al.*, 2011). Several techniques allow the verification of the annual nature of tree rings: cambial marking, ring series cross-correlations, stable isotopes measurements (radiocarbon dating) or studying trees from permanent sample plots with known history (Brienen *et al.*, 2016). However, lots of dendrochronological studies in the Tropics report difficulties in cross-dating and low correlations with climate, causing large uncertainties and many species being discarded (Brienen & Zuidema, 2005; Schongart *et al.*, 2006).

While the application of classical dendrochronological techniques remains challenging in the Tropics due to the less pronounced seasonality and lower tree-ring distinctness, other anatomy-based approaches exist. There is an increasing focus on the sub-annual variations of wood anatomical features which can provide valuable information on trees responses to specific environmental variations (Schweingruber, 2006; Fonti *et al.*, 2010; Battipaglia *et al.*, 2016; De Mil *et al.*, 2017). This approach requires analysing wood anatomy with an increased resolution. While the vessels can be measured thoroughly (Sperry *et al.*, 2006; Choat *et al.*, 2007; Fonti *et al.*, 2010; Sonsin *et al.*, 2012; Zieminska *et al.*, 2013), the tedious nature of fibre measurements made them often overlooked, especially in the tropical angiosperms (Zieminska *et al.*, 2013). New software and plugins allow measuring anatomical features in high resolution (Schneider *et al.*, 2012; von Arx & Carrer, 2014) and thus increase the temporal resolution of the studies. However, they heavily rely on the quality of the sample and surface preparation. Proxies allowing correct and quick estimations of xylem features and their variation are direly needed. Given its recent improvements in resolution and processing time, the potential of densitometry in wood anatomy research is currently explored. Exploring the link between wood density and different anatomical features could provide us a more thorough documentation of their variability and, *in fine*, understand the influence of climate on wood anatomy and, indirectly, on tree growth.

Wood density and wood anatomy

Wood density drivers

Wood density (WD) is a key plant trait, influencing tree performance (Zieminska *et al.*, 2013). It is an integrator variable, determined by chemical and wood anatomical features (Chave *et al.*, 2009; Lachenbruch & McCulloh, 2014). It integrates different aspects of wood mechanical and ecological properties (Chave *et al.*, 2009) and is often used as a proxy to understand different tree species life strategies (Swenson & Enquist, 2007, Zanne *et al.*, 2010). WD is defined as ovendry mass divided by its

volume (at any given moisture content) and can approximate the allocation of carbon by the tree (Plourde *et al.*, 2015).

The xylem is mainly composed of three cell types assuming different interrelated functions: vessels (water transport), fibres (mechanical strength) and parenchyma (nutrient storage and transport). They all have varying structural properties and contribute differently to the overall WD of a tree (Zieminska *et al.*, 2013). While vessels lumens have zero density and should negatively affect WD, several studies have shown only a marginal correlation between the two variables (Martinez-Cabrera *et al.*, 2009; Zieminska *et al.*, 2013). The parenchyma has varying characteristics and has been reported correlated positively, negatively or not correlated with WD at all (Jacobsen *et al.*, 2007; Martinez-Cabrera *et al.*, 2009; Rana *et al.*, 2009; Poorter *et al.*, 2010). Finally, fibres properties influence WD the most (Martinez-Cabrera *et al.*, 2009; Zieminska *et al.*, 2013). All these anatomical features are affected by phylogenetic constraints and vary with environmental variables (Chave *et al.*, 2009).

Although WD is the result of a combination of different anatomical features, its biological meaning remains unclear (Martinez-Cabrera *et al.*, 2009; Zieminska *et al.*, 2013). Although recent studies showed only a marginal correlation between WD and vessels, WD is commonly linked with hydraulic strategies with denser species having greater cavitation resistance and thus growing at more negative water potentials (Gotsch et al., 2010; Lens et al., 2011). Denser woods also tend to be stiffer and more resistant to pathogen attacks (Chave et al., 2009; Romero & Bolker, 2008). They also experience lower stem mortality rates (Poorter *et al.*, 2010).

In general, there is a negative relation between WD and growth rate due to the higher investment in mass per volume during wood formation (Enquist et al., 1999; Muller-Landau, 2004; Nock *et al.*, 2009). Inversely, fast growing species are often associated with the production of wood with low density (Woodcock & Shier, 2002) which provides an advantage in the context of competition for light but reduces tree longevity (Wiemann & Williamson, 1988). Those studies often associate trees growth rates and their life strategies (*i.e.* light-demanding, shade-tolerant, …) which leads, as a result, to the

fact that WD is related to the successional status of a forest (Bastin *et al.*, 2015). Within a tree, also, WD variation is a proxy for tree growth rate. Moya & Filho (2009) showed for *Gmelina arborea* that density peaks corresponded to tree-ring boundaries with latewood cells caused by the dry season in Costa Rica.

WD presents substantial variability at the tree, species and community levels (Muller-Landau, 2004; Martinez-Cabrera *et al.*, 2009; Bastin *et al.*, 2015). This variability has direct impact on carbon stocks estimations because WD is the second best predictor of the biomass of a tree (Chave *et al.*, 2005; Chave *et al.*, 2014) and is often used as an average extracted from global databases such as DRYAD (Zanne *et al.*, 2009). Besides carbon stocks estimations, WD variability can be used in ecology and forest studies (Nock *et al.*, 2009, Plourde *et al.*, 2015). However, the underlying anatomical causes for the observed WD variability are yet to be explored for tropical species (De Ridder, 2013) and to be understood in the context of changing environmental conditions.

Densitometry in tree studies

Besides linking WD to trees' mechanical strength, growth rate or carbon content, several attempts of using WD in dendrochronology were made. Pith-to-bark density profiles were needed and could be obtained with X-ray densitometry, first developed by Polge (1966). The use of X-rays for dendrochronological purposes on tropical trees was first tried by Mariaux (1967). However, delineating tree-ring boundaries with densitometry revealed difficult for tropical species due to vessel and parenchyma patterns inducing a higher variability than in temperate species. Some species, nevertheless, like *Gmelina arborea* allowed densitometric techniques to be used for growth rates and climate-growth studies (Akachuku, 1985; von Schnakenburg et al., 2008; Moya & Filho, 2009).

Recent technological advances significantly increased the analysis resolution and the processing speed. X-ray computed tomography (X-ray CT) allows observing the internal structure of objects in 3D with a resolution up to submicron levels (Van den Bulcke *et al.*, 2009; Dierick *et al.*, 2014) making it a versatile tool for high-resolution anatomical features measurements and tree-ring analyses (Steffenrem *et al.*,

2014). The possibility to study detailed WD micro-variations along the pith-to-bark axis opens new prospects in disentangling the WD into its anatomical components in order to better understand trees reactions to their environment throughout their life.

A better understanding of the biological meaning of WD and linking its variations to the tree's environment would highlight the possibilities of using WD in ecological and dendrochronological studies.

Original contribution and thesis outline

The overall objective of this thesis is to explore the radial variability of wood anatomical features and wood density as well as their intra- and inter-species differences in order to contribute to the development of the techniques of tree growth analysis of the tropical species.

To do so, we studied wood anatomy and wood density of several common tree species spread across three different sites from the Congo Basin, an understudied region (Malhi *et al.*, 2013). Three narrower objectives have been set, each associated to one chapter:

a) To assess the variability of tree-ring distinctness in the Central African tree species and the relationship between wood anatomical features and tree-ring distinctness.
b) To study the relationship between wood density micro-variations and wood anatomical features and to assess the potential of pith-to-bark wood density profiles to serve as proxies for wood anatomy.
c) To develop new techniques of studying past cambial activity by quantifying different trends of wood density variations and to compare them between species and between conspecific individuals growing in contrasted environmental conditions.

This thesis consists of an introductory chapter, three research chapters (Chapters 1-3) and a general discussion. Each chapter focuses on a particular component of wood anatomy in the Tropics: tropical growth-ring boundaries distinctness (Chapter 1), the influence of different anatomical features on

wood density in tropical hardwoods (Chapter 2) and different patterns of pith-to-bark density and anatomy variability under contrasted environmental conditions (Chapter 3). In the introduction, we briefly presented the climate change issue, how it threatens forests and their role in its mitigation. We summarized several knowledge gaps in forest studies and delineated possible research that might help filling these gaps. Wood anatomy is thus explored in the following chapters of this thesis.

In Chapter 1, we explore the diversity of wood anatomical features and their relationship with the distinctness of growth rings of >100 central African tree species. We calculate the proportion of samples with distinct, indistinct and absent growth rings in the hardwood collection of the Royal Museum of Central Africa and describe the most common anatomical features delineating growth-ring boundaries. Furthermore, we compare the growth-ring distinctness between two contrasted sites and between evergreen and semi-deciduous species. We discuss the challenges of defining growth-ring distinctness in the Tropics and introduce the idea of studying pith-to-bark profiles of anatomical variations instead of focusing only on growth-ring width (Fonti *et al.*, 2010).

Based on that premise, in Chapter 2, we study the relationship between wood density and wood anatomy and try to assess the potential of wood density profiles to serve as proxies for wood anatomy. We use recent advances in high-resolution densitometry (Van den Bulcke et al., 2014; De Mil et al., 2016) and image analysis to study the evolution of different anatomical features (fibres, vessels, parenchyma) along with micro-variations of wood density on the pith-to-bark axis for eight tropical species. We discuss our findings and compare them with the previous conclusions on the wood density – wood anatomy relationship. Finally, we discuss the technical challenges to overcome in order to further improve our understanding of this relationship.

In Chapter 3 we use our previous findings on the wood density – wood anatomy relationship and further explore the potential of wood density profiles in tree studies. We study the wood density radial variability of 13 tropical species in three sites located along a precipitation gradient from zero to four dry months per year in order to detect differences of anatomical patterns between different sites. We

derive three descriptors that quantify different trends of density variations. We also use historical records of leaf phenology for these species in the driest and the wettest sites and assess the influence of the pluviometric regimes and leaf phenology on the wood density profiles variability.

Finally, we discuss our work and its contribution to the study of tropical trees growth and suggest further study directions. We end with a conclusion and several take-home messages.

Datasets

- Chapter 1 – Growth-ring distinctness analysis

We browsed the Royal Museum of Central Africa (RMCA) collection of the tropical Congolese wood specimens. The samples were collected across the Congo Basin during the previous studies and stored under form of thin sections of various shapes and sizes. The samples selection was carried out on the basis of the quality of the sections: only the samples with discernible anatomical features were retained for the study. A total of 103 species represented by 321 samples (1 to 11 samples per species) were retained for this study.

Phenological data for each species were extracted from the INERA (Institut National pour l'Etude et la Recherche Agronomiques) observations that took place from 1947 to 1957 in the Biosphere Reserve of Luki (Couralet *et al.* 2010).

- Chapter 2 – The study of the relationship between wood density and wood anatomy

For this study, tree cores of eight species (Table 0-1) were collected with an increment borer (5.15 mm of diameter) during a field mission that took place in the Democratic Republic of Congo (DRC) in July-August 2015. The eight species were selected on the basis of their average wood density and their wood anatomy: the eight species cover a gradient of wood density from 400 to 800 kg/m³ and show various types of anatomical organisations (varying proportions of parenchyma and vessels, large fibre lumina and thin walls to flattened lumina and thick walls). One sample of 3 cm under bark was selected for each species. The selection was based on the quality of the preparation of the sample: no missing pieces, the least possible scratches, no damaged fibres.

While the selected species belong to different guilds and have different leaf phenologies (Table 0-1), this information is not used in our study: we study the relationship between wood density and wood anatomy regardless of the ecology of the species.

- Chapter 3 – The study of the influence of precipitation on wood density variability

Tree cores of 13 species were collected during the same mission as for Chapter 2. These 13 species were selected among the list of species common to the three study sites on the basis of their availability (rare species were ruled out) and their wood anatomy (we selected species with discernible and measurable anatomical features). Wood anatomy was studied on the previously collected samples of the RMCA collection.

The sampling took place in three study sites located along a precipitation gradient in the DRC: Luki (5.39°S 13.4°E), Malebo (2.49°S, 16.50°E) and Yoko (0.33°N, 25.31°E). At each site, ten trees per species were cored, three to four cores per tree. Trees were selected randomly with a minimal diameter at breast height (DBH) threshold set to 20 cm.

In parallel, phenological records from the driest (Luki) and the wettest (Yangambi) site were used. Although Yangambi is not among the sites where sampling took place, its location nearby Yoko and the similarity of precipitation patterns between both sites allow using leaf phenology data from that site. Observations in Luki spanned from 1947 to 1957 (with 1952 missing) while those in Yangambi took place between 1937 and 1956. Observations were weekly in Yangambi and took place every ten days in Luki. The species and the number of trees that were recorded per site are available in Table 0-1.

Table 0-1 : Species studied in the third chapter with their ecological information, the number of observed trees and observation span in each site.

Species	Guild	Leaf phenology	Observed trees (#)		Span of observations (years)	
			Luki	Yangambi	Luki	Yangambi
*Anonidium manii**	SB	E	0	2	-	46-56
*Canarium schweinfurthii**	NPLD	D	16	5	47-57	38-52
Cola griseiflora	SB	E	0	5	-	38-53
*Entandrophragma angolense**	NPLD	D	6	4	47-57	38-52
Gilbertiodendron dewevreii	SB	E	0	5	-	38-56
Macaranga spinosa	LD	E	1	3	47-57	38-53
*Milicia excelsa**	NPLD	D	31	6	47-57	37-56
Ongokea gore	NPLD	E	17	3	47-57	38-52
*Polyalthia suaveolens**	LD	E	50	0	47-57	-
*Pycnanthus angolensis**	NPLD	E	27	4	47-57	37-55
Ricinodendron heudelotii	LD	D	46	3	47-57	37-55
*Staudtia kamerunensis**	SB	E	46	2	47-57	46-56
*Tetrorchidium didymostemon**	LD	E	7	3	47-57	38-55

*The species with the asterisk * were also used in the Chapter 2; SB = Shade bearer, NPLD = non-pioneer light demanding, LD = light demanding; E = evergreen, D = deciduous.*

Study sites

The samples of the second and third chapters were collected during a field mission in three sites located along a precipitation gradient in the Democratic Republic of Congo (DRC; Figure 0-3): the Yoko Reserve (0.33°N, 25.31°E), the Bolobo forests of Malebo (2.49°S, 16.50°E) and the Man and Biosphere Reserve of Luki (5.39°S 13.4°E).

The Yoko reserve is located south of the Congo River, 28 km from Kisangani in the Tshopo province (0.33°N, 25.31°E) and covers an area of 6975 ha (Cassart *et al.*, 2017). The region has an Af climate type following Köppen-Geiger (Peel *et al.* 2007). According to Fayolle *et al.* (2014), the forests in the region belong to the "moist Central Africa" group. Based on the meteorological data collected from the Yangambi station (150 km to the North-West from Yoko) between 1961 and 2009, mean annual precipitation is around 1780, with the months of December-February recording a lower precipitation

around 100 mm (Cassart *et al.*, 2017). A smaller season with less precipitation occurs in June to July. Temperatures vary little throughout the year with a mean of 24.8°C. The region is characterized by a main sandy plateau (altitude 450 m) dissected by a hydrographic network (Cassart *et al.*, 2017). The dominant soil type is classified as Xanthic Ferralsol (WRB, 2014) and is nutrient poor, deeply weathered and acidic. Its plant extractable water capacity is between 8.77 and 11.65 cm (Dunne & Willmott, 2000). The vegetation is dominated by two main old-growth forest types: moist semi-deciduous rainforests with a closed canopy and patches of moist evergreen rainforests forming a continuous canopy with sparse understory (Gilson et al., 1956). The two dominant forest types are the *Scorodephelus zenkeri* Harms mixed forests and *Gilbertiodendron dewevrei* monodominant forests with more than 60% of the canopy level trees belonging to the same species (Connell & Lowman, 1989; Cassart, 2018). Finally, several transition forests and slash-and-burn agriculture areas are located on the west side of the reserve along the main road (Cassart *et al.*, 2017).

The Bolobo forests are located in the region of Malebo, on the edge of the Congo Basin, in the province of Mai-Ndombe (2.49°S, 16.50°E). The sampling area covers an area of approximately 400 km² in the northern part of the Bateke Plateau, with an altitude between 300 and 500 meters above sea level (masl; Bastin, 2014). Precipitation varies between 1500 and 1600 mm/year with a dry season of less than 50 mm/month concentrated around the months of June to August. The average monthly temperature varies around 25°C (Vancutsem *et al.*, 2006). Ferralic Arenosols are the dominant soil types (FAO 2007) with plant extractable water capacity between 4.31 and 6.08 cm (Dunne & Willmott, 2000) which means that trees grow on soils with low fertility and water retention capacity. The landscape is a “forest-savannah” mosaic with a wide diversity of *terra firme* forests, riverine gallery forests, old secondary forests, *Marantaceae* forests, mature forests and old growth monodominant *Gilbertiodendron dewevrei* forests (Bastin *et al.*, 2015). Although local communities manage their environment in a sustainable way, they practice slash and burn agriculture on forest edges. A cattle ranching society is also present and maintains the local savannahs with multiple fires per year.

Nowadays, however, a decrease in the fire regime leads to a recolonization of the savannahs (Bastin, 2014).

The Biosphere Reserve of Luki (Kongo Central Province, 5.39°S 13.4°E) is situated in the Mayombe Forest that covers the southernmost part of Gabon and the western parts of the DRC and the Republic of Congo. It is a semi-evergreen rainforest occurring in a mosaic landscape with patches of savannah and successional forest types on a plateau with an altitude comprised between 500 and 600 masl. The reserve has a typical zonation pattern with a protected area surrounded by a buffer zone where conservation is emphasized and a surrounding transition area (De Ridder, 2013). The forest is composed of deciduous and evergreen tree species in the upper canopy and mostly evergreen species in the understory (Donis 1948; Lebrun & Gilbert 1954; Couralet *et al.* 2010). The dry season lasts from May to September-October. Its intensity, however, is buffered by the proximity of the ocean that maintains the relative humidity constant throughout the year. Temperatures vary between 21°C and 26°C and annual precipitation varies around 1100 mm (De Ridder *et al.* 2013). The soils are Orthic Ferralsols (FAO 2007) with plant extractable water capacity between 6.08 and 7.11 cm (Dunne & Willmott, 2000).

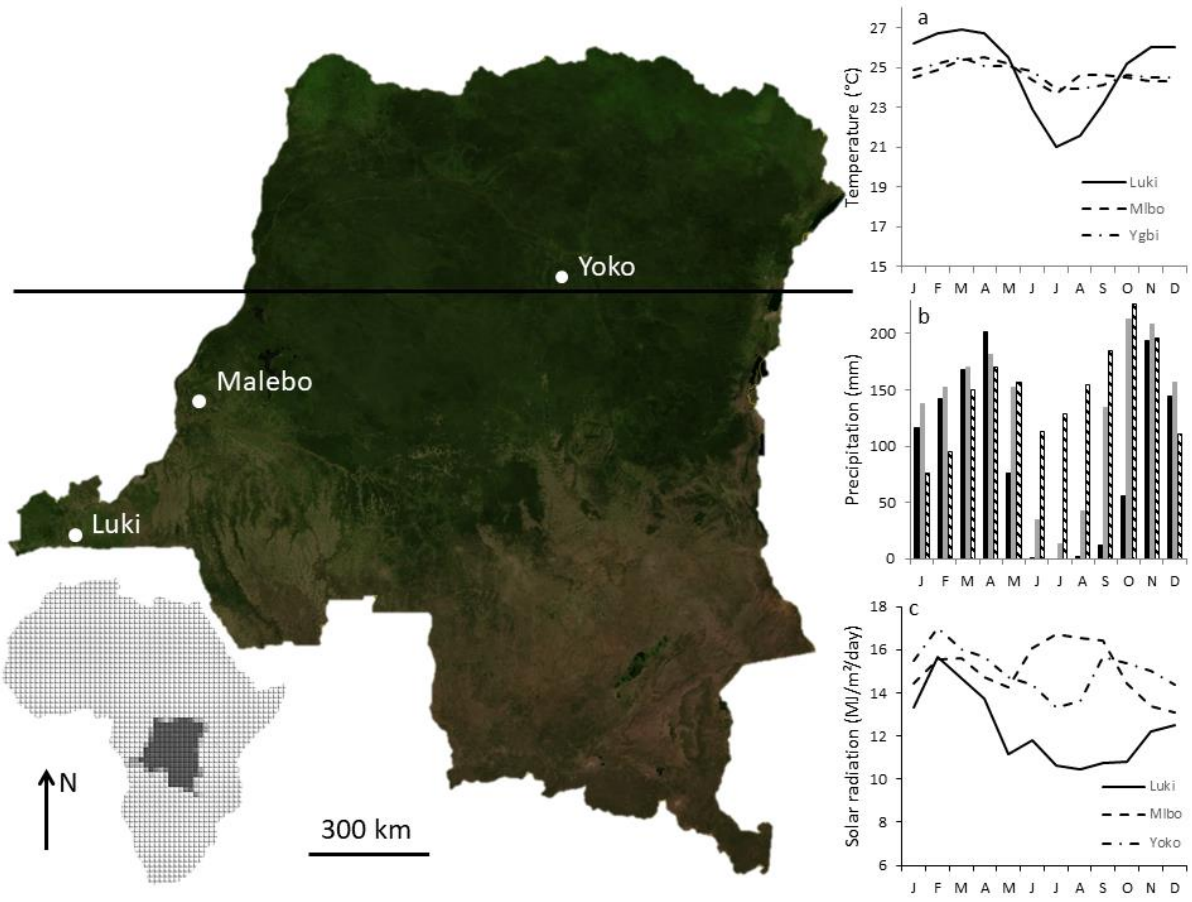

Figure 0-3 : The location of the three study sites and their respective temperature (a), precipitation (b) and solar radiation (c) annual patterns. Precipitation bars: Luki (black), Malebo (grey) and Yoko (striped). The black line represents the equator. Data: Worldclim2 (Fick & Hijmans, 2017).

Chapter 1: The variability of growth-ring distinctness in the Tropics

Chapter 1: The variability of growth-ring distinctness in the Tropics

Title

Growth-ring distinctness and boundary anatomy variability in tropical trees.

Authors

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Authors contribution

- Paper conception: Tarelkin, De Cannière, Beeckman
- Datasets: Tarelkin, Delvaux, De Ridder, Beeckman
- Laboratory work: Tarelkin, El Berkani, De Ridder
- Analyses: Tarelkin, De Ridder, De Cannière
- Major review: Tarelkin, De Ridder, De Cannière, Beeckman
- Minor review: all the co-authors

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Abstract

The phenomenon of distinct, absent or indistinct growth rings is a highly variable feature used for wood identification and a wide range of tree-ring studies. Causes for its variability are not yet fully understood. There is also a lack of consensus within the scientific community about how distinct and indistinct tree rings should be defined and classified. In this study, we use a selection of 321 samples to explore the anatomy and distinctness of growth-ring boundaries of 103 Central African rainforest species and to assess the influence of the site and leaf phenology on growth-ring distinctness and anatomy. We observed a high variability of growth-ring boundaries anatomy and distinctness within and among individuals and species. The most common anatomical features driving growth-ring distinctness are flattened fibres, marginal parenchyma and distended rays. No significant difference in growth-ring distinctness was detected between the two studied sites or between evergreen and semi-deciduous species. Current IAWA definitions of growth-ring distinctness appear too subjective and poorly adapted to the tropical context. An approach allowing to reduce the subjectivity in the description of growth-ring boundaries is proposed.

Key words: growth rings; Democratic Republic of the Congo; wood anatomy; phenology.

Introduction

Growth rings are a wood anatomical feature familiar to everybody involved in wood research. The first observations of growth rings with the unaided eye were done by Theophrastes (McBride, 1983) and Da Vinci (Studhalter *et al*., 1963). Van Leeuwenhoek was the first to study growth rings in temperate trees under a microscope (Schierbeek, 1959). He also made microscopic observations of tropical *Diospyros* wood (Baas, 1982; Baas & Vetter, 1989) and noted the absence of distinct growth rings. Presence or absence of distinct growth rings is the start of many dichotomous identification keys for commercial timbers (IAWA Committee, 1989) whereas in fossil wood, it is also a criterion to characterize the climate of the geological era where the fossils originate from (Francis & Hill, 1996).

Tree rings can reveal a lot of information on tree and forest dynamics (Brienen & Zuidema, 2006), tree age (Worbes & Junk, 1999) or help reconstructing past climate (Schongart *et al.*, 2004). However, tree-ring studies in the Tropics can present specific challenges: cambial activity can remain constant throughout the year, leading to a reduced variability of wood anatomical features and decreasing tree-ring distinctness (Groenendijk *et al.*, 2014). However, evidence suggests that tree-ring distinctness is variable within species under contrasting environmental conditions (Moya & Filho, 2009). Within site variability in growth-ring distinctness occurs as well, for example between deciduous and evergreen species (Lisi *et al.*, 2008).

Growth-ring formation is triggered by a succession of favourable and unfavourable growing conditions (Schweingruber, 1996; Whitmore, 1998). The cambial response of trees varies mainly with the environment, the species and the age of a tree (De Ridder *et al.*, 2013; Fonti *et al.*, 2010; De Micco *et al.*, 2016). Besides, a variety of wood anatomical structures defining growth rings are reported. This complexity leads to different 'levels' of growth-ring distinctness (Schweingruber, 1996). For example, Fahn (1962) recognised five types of growth-ring boundaries in trees and shrubs from Israel, essentially based on their distinctness. The first type shows growth rings, the middle three types "more or less" distinct growth rings and the fifth does not show growth rings at all. Carlquist (2001) described 15

growth-ring boundaries types and Worbes (2010) reduced them to four basic types. Next to these classifications, many other studies in temperate and tropical regions use their own classification, mostly directed towards the applicability in dendrochronological studies (Tarhule and Hughes, 2002; Schweingruber *et al*., 2006; Sonsin *et al*., 2012; Groenendijk *et al*., 2014).

The most used and well-known definition is proposed by IAWA in a list of features designed for hardwood identification (IAWA Committee, 1989). It defines distinct growth-ring boundaries by an abrupt structural change at the boundary, usually including change in fibre wall thickness and/or radial fibre diameter. In addition, six other growth-ring boundaries markers, often co-occurring with changes in fibre morphology are listed. IAWA Feature 1 is reserved for species showing distinct growth rings with an abrupt structural change at the growth-ring boundaries, while Feature 2 is for indistinct growth rings, with a more or less gradual change in cell characteristics on a radial axis, and absent rings. However, the classification between Features 1 and 2 largely depends on the judgment of the individual wood anatomist, often biased by his/her experience. Cherubini *et al.* (2013) used one olive branchwood sample and circulated it for growth-ring analysis to different laboratories. The number of counted growth rings varied considerably among the experts showing the subjective character of growth-ring distinctness. Especially under tropical circumstances there is a high level of growth-ring distinctness variability and uncertainty (Wils *et al*., 2009). Thus, the necessity of a clear and at the same time broad definition of growth-ring distinctness is obvious in order to construct databases on ring characteristics for different species and individuals in different environments.

In this study, we explore the anatomical diversity of growth-ring boundaries of 103 tropical species with particular attention to the relationship between the ring boundary anatomy and distinctness. We also assess the influence of leaf phenology on the distinctness of growth-ring boundaries and their variability between two different sites. The following questions are addressed: a) does the IAWA definition of growth-ring distinctness reflect the diversity and complexity of tropical trees anatomy and b) does growth-ring distinctness vary in contrasted environments and between different leaf

phenologies? We expect to observe higher growth-ring distinctness for semi-deciduous species and for individuals from Luki.

Materials and methods

Use of existing collections and database

Wood collection of the RMCA

The Royal Museum of Central Africa (RMCA) collection of tropical Congolese wood specimens was browsed and 321 high-quality microtome sections belonging to 103 different species (1 to 11 samples per species) were selected (Annex 1). They were observed under the microscope (Olympus BX60) and the anatomy of growth rings boundaries was described. If all the fibres on both sides of the ring boundary could be assigned to the n^{th} or the n^{th+1} ring, they were classified as distinct; if they couldn't, they were classified as indistinct. Some samples did not show any visible growth ring, in which case rings were marked absent. To assess the intra-species variability of growth-ring distinctness, we counted the number of species with constant (only distinct, indistinct or absent) or with variable (distinct AND indistinct, distinct AND absent, indistinct AND absent, all the three) growth-ring distinctness. Growth rings were then tested for the presence or absence of several features such as abrupt changes in fibre or vessel dimensions or parenchyma frequency variation (Table 1-1, illustrated in Figure 1-1).

Table 1-1 : List of anatomical features used to describe growth-ring boundaries.

Abbreviation	Anatomical feature
AC_FW	Abrupt change in fibre wall thickness
AC_FD	Abrupt change in radial fibre diameter
GC_FD	Gradual change in radial fibre diameter
GC_VD	Gradual change in vessel diameter
VDV	Vessel density variation
MP	Marginal parenchyma present
PDV	Parenchyma bands density variation
DR	Distended rays

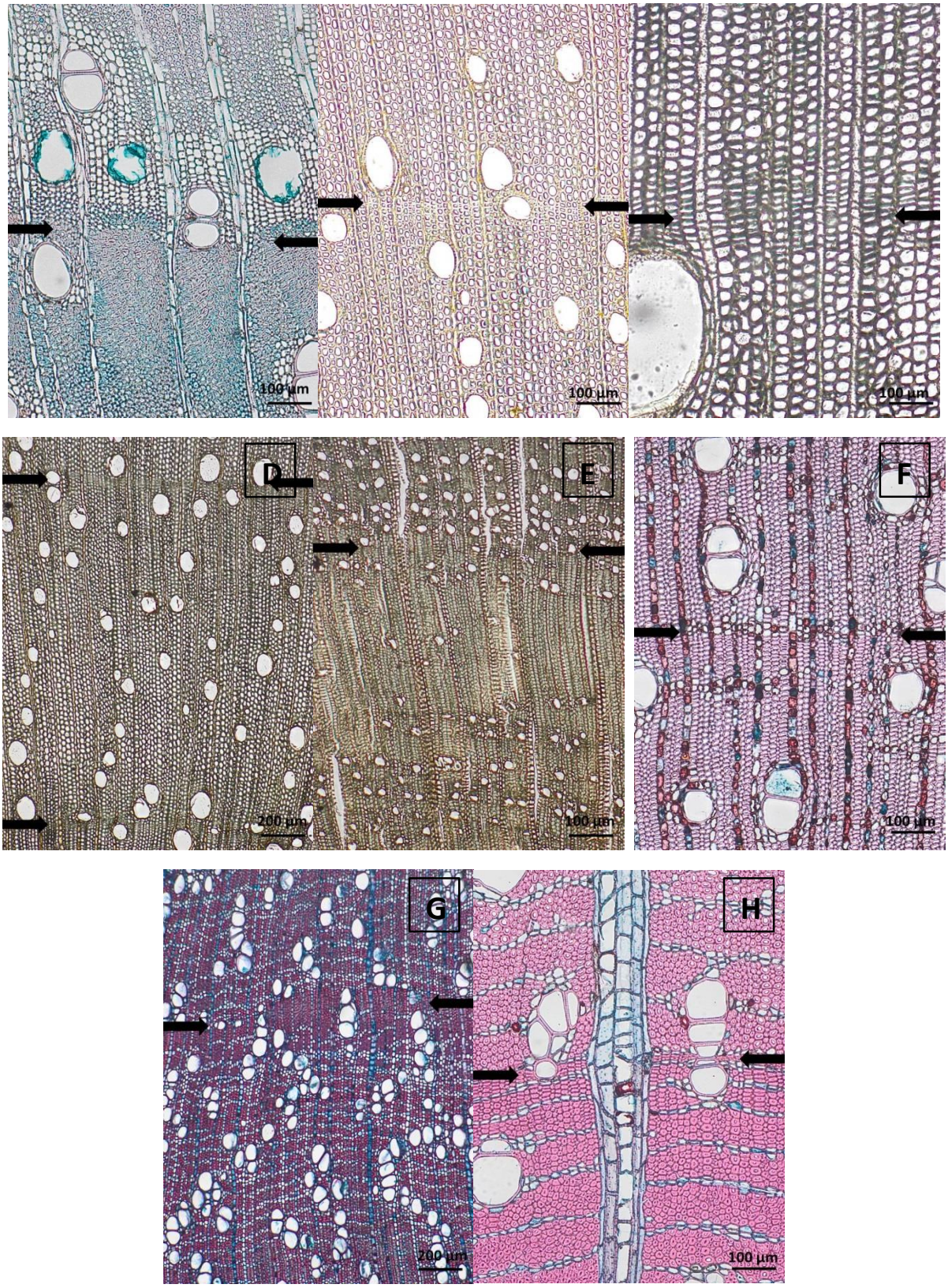

*Figure 1-1 : Illustrations of the studied anatomical features. A: Abrupt change in fibre wall thickness (*Copaifera mildbraedii*). B: Abrupt change in radial fibre diameter (*Agelaea pentagyna*). C: Gradual change in radial fibre diameter (*Ricinodendron heudelotii*). D: Gradual change in vessel diameter (*Agelaea pentagyna*). E: Vessel density variation (*Dichapetalum madagascariense*). F: Marginal parenchyma present (*Trichilia rubescens*). G: Parenchyma bands density variation (*Synsepalum subcordatum*). H: Distended rays (*Polyalthia suaveolens*). Arrows indicate growth-ring boundaries.*

Phenology

Phenological data for each species were extracted from the INERA (Institut National pour l'Etude et la Recherche Agronomiques) observations that took place from 1948 to 1957 in the Biosphere Reserve of Luki. Two categories were created: evergreen and semi-deciduous. Species were classified as semi-deciduous if one or several individuals were observed leafless at least once during the observation period (Couralet *et al*., 2010) and evergreen if no leaf shedding was observed. This classification was possible on a subset of only 51 species (23 semi-deciduous and 28 evergreen) as no information was available on the 52 others in the INERA database.

Study sites

The RMCA collection samples mostly originate from the Democratic Republic of the Congo (DRC). The sites retained for this study show contrasting rainfall regimes, which allows exploring the influence of precipitation seasonality on tree ring distinction. They are located in the Biosphere Reserves of Yangambi (0.46°N 24.28°E) and Luki (5.39°S 13.4°E). Annual precipitation is highest in Yangambi (around 1700 mm) and decreases to around 1200 mm in Luki.

The Biosphere Reserve of Yangambi is located on the northern bank of the Congo River, just north of the equator in the Oriental Province. The region has an Af type tropical rainforest climate (following Köppen-Geiger; Peel *et al*., 2007). The Yangambi region receives an annual precipitation of 1762 mm (1961 - 2012) with, on average, two to three months with lower monthly precipitation around 100 mm, during December – February (Figure 1-2). A smaller season with less precipitation occurs in June to July. Temperatures are fairly constant throughout the year with a minimum of 24.2 °C in July and a maximum of 25.5 °C in March. Vegetation in the reserve is characterized by both old-growth and disturbed forests (Gilson, 1956). Forest disturbance is mainly caused by slash-and-burn agricultural activities followed by abandonment allowing forest regeneration (Kearsley, 2013).

The Biosphere Reserve of Luki is situated in the Lower Congo Province at an altitude of < 300 m above sea level, in a semi-evergreen rainforest occurring in a mosaic landscape with patches of savannah and

successional forest types. The dry season lasts approximately four to five months, from May to September-October. The proximity of the ocean, however, buffers the intensity of the dry period with relative humidity more or less constant throughout the year. Average temperatures are around 26°C and annual precipitation varies around 1100 mm (De Ridder *et al*., 2013).

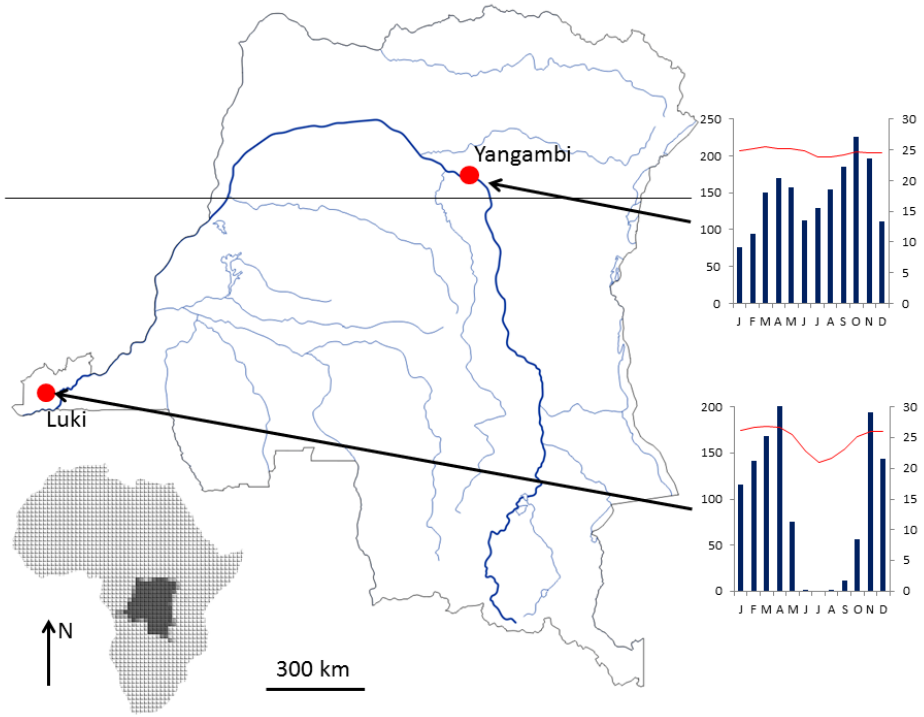

Figure 1-2 : Geographical location of the two study sites with their climate diagrams (monthly precipitation left, average day temperature (red line) right. The horizontal line represents the equator.

Data analysis

Each observed growth ring was described by the presence/absence of eight different anatomical features (Table 1-1) and a score of respectively 1 or 0 assigned per feature. The different combinations of the anatomical markers of growth-ring boundaries were counted and their abundance calculated. We then selected the most abundant configurations of growth-ring boundaries that add up to 75% of the observed rings and described the association of different features and their link with growth-ring distinctness.

The distinctness of each growth-ring was described with 0 for absent rings, 0.5 for indistinct rings and 1 for distinct rings. These values were then averaged at the individual level to test the site effect and at the species level to test the phenology effect. To test the effects of the site on ring boundary distinctness, we constructed a mixed effects model with the species as the random effect. To study the effects of leaf phenology, we compared evergreen and semi-deciduous species with the Kruskal-Wallis test. The tests were performed on subsets composed of species that presented samples from both Luki and Yangambi (46 species) or species with known information on their leaf shedding behaviour (51 species). All these analyses were performed with R software (R Core Team, 2013).

Results

Growth-ring boundaries variability

321 samples sorted in 103 species from the RMCA collection were analysed with, on average 3.2 samples per species. 28% of samples presented distinct growth rings, another 28% presented indistinct growth rings while 44% showed no growth rings at all. Figure 1-3 shows the intra-species variability of growth-ring distinctness.

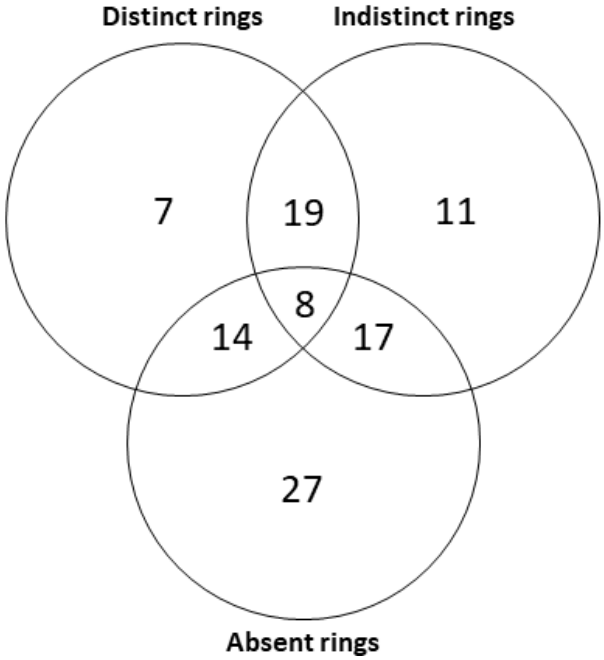

Figure 1-3 : Intra-species variability of growth-ring distinctness. Each number represents the number of species showing samples either with the same level of ring distinctness or showing different samples with different levels of ring distinctness.

In total, 399 ring boundaries were observed and 42 different configurations of boundary markers were counted. Twelve configurations represent 75% of the observations (Table 1-2). The most common feature encountered in growth-ring boundaries is the marginal parenchyma, followed by fibres diameter variations and distended rays. Moreover, different associations of these features are quite common and represent the four most encountered configurations of growth-ring boundaries, accounting together for more than 40% of the observed rings. Inversely, changes related to vessels (diameter, vessel density) are among the less frequent.

The features increasing growth-ring distinctness are a combination of abrupt changes in fibres, marginal parenchyma and distended rays. However, marginal parenchyma is also encountered in indistinct rings, meaning that, as such, this feature doesn't make ring boundaries distinct. Abrupt changes in fibres increase growth-ring distinctness. Distended rays, to a lesser extent, also drive growth-ring distinctness. The variations of parenchyma bands or vessel density, although helping growth-ring boundaries detection, are only sufficient to create indistinct growth rings. Finally, no abrupt changes related to vessels were observed and gradual changes in vessel diameter were rare in our sample set. All growth-ring boundaries descriptions are available in Annex 1.

Table 1-2 : List of the most common configurations of growth-ring boundaries anatomical markers. The right column indicates the proportion of ring boundaries with that anatomical configuration.

AC_FW	AC_FD	GC_FD	GC_VD	VDV	MP	PDV	DR	Distinct	Indistinct	%
	1							1		17
	1				1			1		10
					1		1	1		9
	1				1		1	1		7
					1	1			1	6
				1					1	5
					1				1	4
		1							1	4
1					1		1	1		4
1							1	1		3
	1				1	1		1		3
	1						1	1		3

AC_FW: abrupt change in fibre wall thickness; AC_FD: abrupt change in fibre diameter; GC_FD: gradual change in fibre diameter; GC_VD: gradual change in vessel diameter; VDV: vessel density variation; MP: marginal parenchyma; PDV: parenchyma density variation; DR: distended rays.

The variability of growth-ring distinctness between different sites

Samples from both Luki and Yangambi were available for only 46 species in the studied sample set. The average growth-ring distinctness per site is represented in Figure 1-4: in both sites, it is close to 0.5, meaning that the number of species with distinct rings is equivalent to the number of species without visible growth rings. Intra-specific variability is relatively close between both sites: 12 species with variable distinctness in Luki and 10 in Yangambi. The p-value of the mixed model is 0.49, meaning that there is no significant difference in growth-ring distinctness between Luki and Yangambi in our sample set.

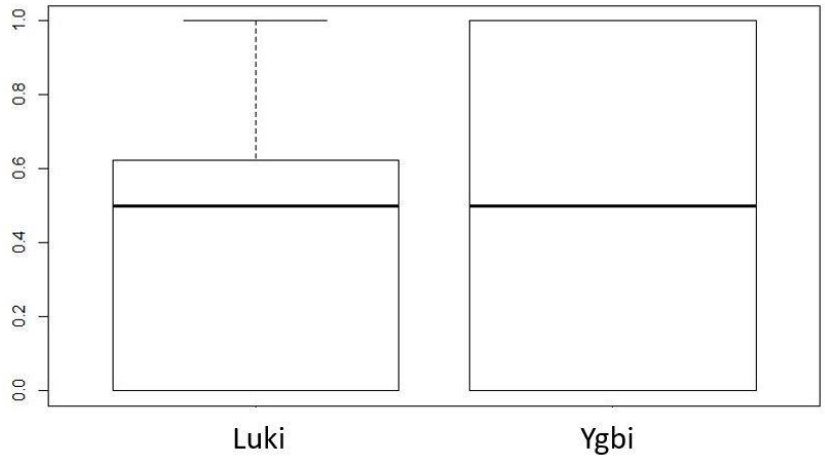

Figure 1-4 : Boxplot of growth-ring distinctness scores of 46 species in Luki and Yangambi. The scale from 0 to 1 indicates the average growth-ring distinctness per species: 0 = no observed growth ring, 1 = all growth rings are distinct.

The difference of growth-ring distinctness between semi-deciduous and evergreen species

Only 51 species were used for this analysis due to the lack of information about leaf-shedding behaviour of other species. The average growth-ring distinctness for each leaf-shedding behaviour is represented in Figure 1-5. The average growth-ring distinctness of evergreen species appears slightly lower than semi-deciduous ones. However, the p-value of the Kruskal-Wallis test is 0.61, meaning that there is no significant difference in growth-ring distinctness between evergreen and semi-deciduous species in our sample set.

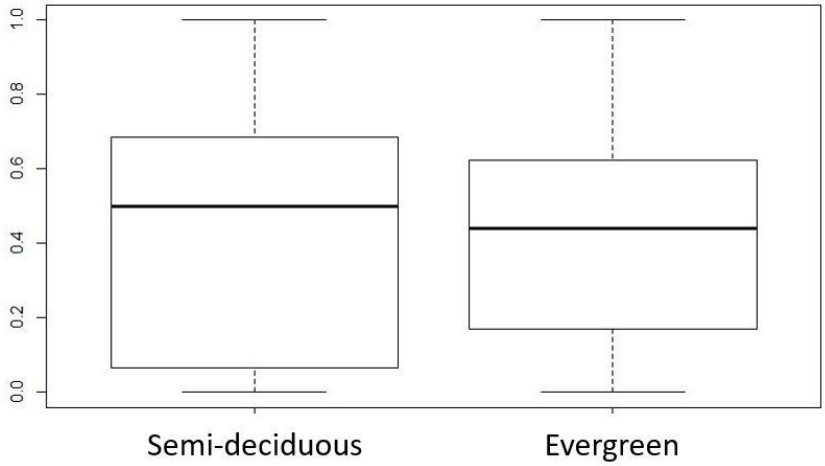

Figure 1-5 : Boxplot of growth-ring distinctness scores for 23 semi-deciduous and 28 evergreen species. The scale from 0 to 1 indicates the average growth-ring distinctness per species: 0 = no observed growth ring, 1 = all growth rings are distinct.

Discussion

We observed variation in growth-ring boundaries anatomy between species, within species and even within individuals (Annex 1). These observations are in accordance with Fichtler and Worbes (2012). So far, growth-ring distinctness was mainly described at the species level without acknowledging possible intra-species variation (Worbes, 1999; Groenendijk *et al.*, 2014). Our study highlights the high level of variation of growth-ring boundary anatomy and distinctness in the tropics and raises questions about the drivers of this variation. Here, we try to address some of these questions.

Growth-ring boundaries and anatomical variability

Four types of growth-ring boundaries are commonly described in the litterature: a) flattened fibres with thickened cell walls, b) marginal parenchyma bands, c) periodically recurring parenchyma and fibre bands of different width and d) varying frequency and diametres of vessels (Worbes, 1989). We showed in our study that these features are often associated and that some features tend to associate more between them than others. Marginal parenchyma seems to be the most commonly associated feature with others (abrupt fibre changes, vessel density variations, distended rays). Marginal parenchyma's function is related to starch storage (Carlquist, 1988). Gourlay (1995) suggested that it could serve supporting rapid flushes of growth, flowering and fruiting once the favourable growth conditions return, which could explain the relative frequent occurrence of this feature at growth-ring

boundaries. Local distentions of the radial parenchyma might serve the same goal of favouring rapid growth resumption.

Only a small number of species of our sample set showed vessel density variation. Ring porosity is thought to favour rapid water conduction during wet seasons and form more drought-resistant wood during dry seasons (Gilbert, 1940). However, the absence of dry season in Yangambi and the relatively high air humidity in Luki all year round may provide insufficiently dry conditions to favour the establishment of species with varying densities of vessels. More generally, ring porosity is less frequent in the Tropics than in temperate regions (Gilbert, 1940). Our results show that while ring boundaries defined by vessel density variations are easy to spot, they are usually indistinct. It needs an association with other features such as marginal parenchyma or abrupt changes in fibres to obtain distinct rings (Table 1-2).

The observed diversity of growth-ring boundaries anatomy mirrors the more general diversity of wood anatomical structures which is driven by the species richness in the Tropics (Wheeler *et al.*, 2007; Fichtler & Worbes, 2012). We believe that given the high variability of tropical growth-ring boundaries anatomy and the frequent occurrence of features other than fibres (marginal parenchyma, rays, …), definitions and databases of growth-ring distinctness should acknowledge that variability. Growth-ring distinctness and anatomy descriptions could be complemented with the information about the geographic area of the study, which would allow assessing the evolution of growth-ring distinctness in function of geographical gradients (e.g. like it is done for other traits in databases such as CoForTraits; Benedet *et al.*, 2015).

Differences of growth-ring distinctness between different sites and leaf phenologies

A high-level of intra-species and even intra-individual variability of growth rings anatomy was observed. High levels of wood structure variability have been reported for tropical species in the past (Worbes, 1988) and are supposed to be driven by varying environmental and developmental factors

(Fichtler & Worbes, 2012). In this section, we explore the influence of leaf phenology and the site effect on growth-ring distinctness variability.

Sites

Luki and Yangambi differ in terms of total annual precipitation, the length of the dry season and temperature (Figure 1-2). The precipitation during Luki's dry season is below the critical threshold of 50mm per month, which is expected to impact cambial activity and influence growth-ring distinctness (Worbes, 1995; Worbes, 2010; Zanne *et al*., 2010, Fichtler & Worbes, 2012).

However, although some species showed differences between both sites, no significant difference in growth-ring distinctness was detected between Luki and Yangambi overall. Although the studied sites differ in terms of annual rainfall and the length of the dry season, other factors such as a constantly high relative humidity in Luki buffer the intensity of the dry periods (De Ridder, 2013). According to Borchert (1999), plants react to drought but responses vary widely among trees and their annual development is not well synchronized by climatic seasonality. Moreover, other studies have reported a greater variability between individuals at one site rather than between different sites (Fichtler & Worbes, 2012). Although the number of individuals in the present study is limited, our observations appear to be in line with Fichtler and Worbes' findings. This may indicate that cambial activity is more sensitive to climatic variability from year to year or to micro-environmental factors rather than to the average climate conditions per site.

Leaf phenology

According to Borchert (1999), leaf phenology and cambial activity are correlated with each other in tropical dry climates and get progressively uncoupled with the increase in precipitation. This suggests that growth-ring distinctness is linked to leaf phenology (Worbes, 1999; Brienen & Zuidema, 2005). Based on previous research (Worbes, 1999; Lisi *et al.*, 2008; Nath *et al.*, 2016), we expected to observe a higher growth-ring distinctness among semi-deciduous species.

However, we didn't detect significant differences between semi-deciduous and evergreen species that compose our sample set. Moreover, both groups showed varying levels of growth-ring distinctness, ranging from species with totally distinct rings to species with no visible rings at all. Our observations support previous research that states that not all deciduous species form distinct rings (Worbes, 1999) and that although evergreen species don't shade leaves, they modulate their cambial activity in function of the environmental conditions (Borchert, 1999; O'Brien *et al.*, 2008).

Remaining issues and needs for future research on growth-ring distinctness

Two distinct goals were pursued during this study: exploring the variability of growth-ring boundaries anatomy and assessing the difference of growth-ring distinctness between different leaf phenologies and sites. Our sample set contains many individuals representing a considerable number of species, allowing thus to explore thoroughly the intra- and inter-species anatomical variability. However, some limitations exist. The samples are often variable in size with little to no metadata; some of them are cubes of *ca.* 2 x 2 x 2cm with no indication of their position within the tree. Although the absence of distinct growth rings in those samples is valuable information about anatomical patterns and growth trends, it does not provide the information about possible growth rings in other parts of the trunk. Moreover, even if the described growth ring is distinct, we describe only a part of its variability and know little about its anatomy elsewhere. This is especially true if only few samples are available. To avoid the lack of representativeness, we recommend studying growth rings on pith-to-bark cores or complete disks.

While we could not highlight a significant influence of the site or leaf phenology effect, it is important to keep in mind that our study is an exploratory approach of different trends of a large, but heterogenous, dataset. It gives us a first insight of the possible differences in cambial activity of tropical trees in two different sites but there is no precise information about the response of a single species to different climatic conditions. In several species, only few individuals per site were available, hampering thus a more thorough analysis of within-species response to the site. A more adapted

sampling design with more replications per species and per site is necessary to effectively tackle such analyses.

Our observations suggest that it might be important to redefine the concept of growth-ring distinctness in the tropical trees. Current IAWA definitions of growth-ring distinctness don't discriminate between temperate and tropical species and use vague terminology such as the adverb "usually" when referring to changes in fibre wall thickness and/or radial diameter (IAWA committee, 1989). Moreover, the meaning of "distinct" and "indistinct" is qualitative and might vary from one scientist to another (as is the separation of "indistinct" and "absent" as judged from the illustrations in the Hardwood List). In several cases in our sample set, boundaries were defined by parenchyma patterns, vessel organization or distended rays associated with more-or-less gradual variation in fibre anatomy. It is challenging to describe the distinctness of such ring boundaries using current IAWA's definitions. During this study, we segregated distinct and indistinct growth rings based on the possibility to assign each cell to the n^{th} or n^{th+1} ring. While the utility of such an approach in dendrochronological protocols is debatable (some indistinct rings can still be used in dendrochronology), this approach helps overcoming the subjectivity issues linked with the IAWA's definitions. Other quantitative techniques for assessing growth-ring distinctness could also be elaborated (e.g., a measurement of the distance between consecutive parenchyma bands, high-resolution densitometry, …). Schweingruber *et al.* (2006) propose a more complete description of growth-ring distinctness with attention paid to the possibility to cross-date rings and conduct dendrochronological studies. A measure of the degree of distinctness (*e.g.*, based on the amplitude of variation of radial fibre diameter between fibres before and at the boundary of a ring) could be implemented to measure the sensitivity of trees to variable environmental conditions. This can complement the previously researched features like ring width (Worbes, 1999) or the variation in vessel pattern (Fichtler & Worbes, 2012).

Furthermore, we believe that ring width measurement is just one of many other methods of studying past and present tree growth. The development of computing and analysis power of the last decades led to the development of new methods that emphasize continuous analyse of anatomical features (vessels, fibres) from pith to bark (Fonti *et al.*, 2010; Fichtler & Worbes, 2012; von Arx *et al.*, 2014; De Mil *et al.*, 2017). Focusing not only on growth rings but also on wood anatomy between them will help increasing the resolution to sub-annual levels and more thoroughly understand the link between cambial activity and the environmental conditions (Fichtler & Worbes, 2012).

Conclusion

In this study we browsed the RMCA database to explore the anatomical variability of growth-ring boundaries of tropical species and their distinctness. We showed that fibres and parenchyma are the features the most frequently involved in the delimitation of distinct growth rings. We also observed intra- and inter-individual variability of growth rings anatomy and growth-ring distinctness. Our results suggest that current definitions of growth-ring distinctness don't grasp the complexity of tropical trees anatomy. Unlike previous research, we couldn't detect a significant influence of leaf phenology or site effect on growth-ring distinctness. A range of issues related to the nature of the studied samples hampered the analysis and an improved study design can be implemented.

Acknowledgments

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Chapter 2: The wood density – wood anatomy relationship of eight tropical species

Chapter 2: The wood density – wood anatomy relationship of eight tropical species

Preface Chapter 2

In Chapter 1 we showed the high variability of growth-ring distinctness within and between tropical species. We concluded that our current definitions don't grasp the complexity and the variability of wood anatomy and growth rings in the tropics. Moreover, focusing only on species forming distinct tree rings undermines our chances of studying and understanding tree growth of important species that don't form distinct tree rings.

On the other hand, intra-annual density fluctuations (IADFs) increasingly draw attention (De Ridder *et al.,* 2013; De Mil, 2017). These IADFs can potentially be used to study tree growth patterns with higher temporal resolution than classical dendrochronology since they allow studying cambial activity patterns at sub-annual levels.

However, extensive research is needed in order to understand IADFs formation and their relationship with the climate and correctly implement them in tree-growth studies. It is vital to describe the anatomical drivers of wood density fluctuations and to link them to different climatic events in order to understand trees reactions.

Our work takes place in the framework of the study of the relationship between wood anatomy and wood density. In the following chapter, we try to disentangle the effects of different xylem features on wood density and propose an automated method of measuring different xylem features in order to tackle the issue of tediousness of anatomical measurements.

Title

Varying contribution of wood anatomical features to wood density profiles of eight species of the Congo Basin.

Authors

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- Paper conception: Tarelkin, De Mil, Van den Bulcke, Beeckman, De Cannière
- Samples collection and preparation: Tarelkin, Me Mil, Van den Bulcke
- Analyses: Tarelkin, De Mil, Hahn, Debeir, Van den Bulcke
- Major review: Tarelkin, De Mil, De Cannière, Van den Bulcke
- Minor review: all the co-authors

Abstract

Background and aims

Wood density reveals a tree's life strategy and productivity, and, when measured from pith to bark via detailed profiling, reflects the pattern of wood anatomical variations through time. Density profiles are rarely defined for wood of tropical angiosperm species, which consists of a complex combination of vessels, parenchyma and fibres. The variations of these features along the pith-to-bark axis are promising in tree growth and its relationship with climate studies. Here, we aim at linking these features to the corresponding density profiles of tropical trees, in order to assess the potential of wood density profiles to serve as proxies for wood anatomy.

Methods

Cores of eight hardwood species from the Congo Basin were scanned with X-ray Computed Tomography. The outermost 3 cm of these cores were used to semi-automatically measure major wood anatomical features such as vessels lumina, parenchyma and fibre area percentage were measured via the machine learning Weka segmentation, whereas fibre wall and lumina widths were measured via a newly developed semi-automated fibre recognition, along a radial gradient on surfaced wood segments. A Gaussian linear mixed regression model was used to estimate the relative contribution of each anatomical feature to the density variance, with a species effect on slope and intercept of the regression.

Key Results

The automated approach of both Weka segmentation for tissue percentages, and semi-automated fibre recognition allows to measure fibre wall and lumina fractions in a less tedious way. The model explains most of variation (92 %), with 65 % of the densitometric profile explained by the three features. The remaining 27 % are explained by species as a random effect. Density profiles mostly

represent variations of fibre lumina and wall fractions from pith to bark, for these eight tropical species.

Conclusions

Pith-to-bark profiles can serve as a starting point for estimating wood anatomical variations. Given the large variation between species, future work should further expand these anatomical features analysis from pith to bark to larger number of species and individuals per species.

Keywords

Wood anatomy, X-ray CT densitometry, tropical forests, Congo Basin, fibres, vessels, parenchyma, wood density, specific gravity

Introduction

Wood density is an integrating variable (Chave *et al.*, 2009) and a property defined by chemical and anatomical traits (Lachenbruch & Mcculloh, 2014). Besides applications such as wood quality assessment and materials technology, wood density is assessed in the framework of ecology and carbon dynamics within and across species, taxa and across continents (Baker *et al.*, 2004).

Wood density can vary considerably from pith to bark (Hietz *et al.*, 2013; Bastin *et al.*, 2015) and most variation occurs between ring boundaries due to seasonal dynamics of wood formation (Bouriaud *et al.*, 2005), which is of particular importance in climate reconstructions and studies on the impact of climate change on trees. Maximum latewood density is, for instance, used to reconstruct summer temperature (Briffa, 1998), and earlywood is being explored as well (Björklund *et al.*, 2014) to improve climate reconstructions based on conifer wood.

To study intra-annual patterns of density variation, high-resolution density profiles are needed. This resolution can be obtained with blue intensity (Björklund et al., 2017) or traditional X-ray densitometry (Polge, 1966). X-ray CT microdensitometry is a technique that allows obtaining 3D density volumes, that can be converted to large datasets of density profiles (Bergsten *et al.*, 2001; De Mil *et al.*, 2016), with resolution ranging from submicron level (Van den Bulcke *et al.*, 2009) to coarser resolutions to perform tree-ring analysis (Steffenrem *et al.*, 2014; Van den Bulcke *et al.*, 2014). Density profiles were created for tropical angiosperm species shortly after the establishment of X-ray densitometry (Polge, 1966) for fast growing species such as *Aucoumea klaineana* (Mariaux, 1967) and *Terminalia ivorensis* (Nepveu, 1976), and more recently in semi-arid regions (Pagotto et al., 2017) in order to delineate ring boundaries or to assess wood quality. However, these profiles never explicitly quantitatively related wood density and wood anatomy.

The relation between wood density and anatomical features is straightforward for coniferous species: tracheid size lumina and the cell wall width of tracheids determine the density variation in a ring (Björklund *et al.*, 2017). However, for angiosperms the underlying anatomical signal is more variable

due to the complex combination of vessels, axial and ray parenchyma and fibres that vary within the tree ring to a certain extent. Moreover, in the tropics, there is often a high level of intra- and inter-ring variability of anatomical features. Hence, for an angiosperm tree, the main xylem functions such as water transport and storage, mechanical stability and storage of photosynthates (Badel et al., 2015), are carried out by different anatomical components. Although some of these features are multifunctional (Beeckman, 2016), it is important to quantify them in function of (i) their contribution to wood density and (ii) their potential use for dendroclimatic analysis.

Anatomical decomposition of angiosperm wood has been reported across species (Ziemińska *et al.*, 2013, 2015), yet decomposing a pith-to-bark density profile into its anatomical components has rarely been done. Attempts to locally disentangle intra-annual profiles into vessels, parenchyma and fibres have been reported for the temperate species *Quercus petraea* (Guilley *et al.*, 2002) and in some tropical ones (Roque & Tomazelo-Filho, 2007), but a plethora of angiosperm tropical species with a wide range of wood anatomical features variability (Fichtler & Worbes, 2012) and life strategies remain understudied.

Identifying and manually measuring a statistically relevant number of anatomical features can be tedious and time consuming, preventing the scientific community from gathering large databases on anatomical variability on a pith-to-bark axis. In this study, we aim at further exploring the link between wood density and wood anatomy for, ultimately, bridging the more tedious quantitative wood anatomy with the more high-throughput method of X-ray CT microdensitometry. To do so, we compare wood anatomical measurements to high-resolution X-ray CT profiles of eight common tropical species with varying densities and wood anatomies. Two research questions are defined: a) which anatomical feature influences wood density the most and b) can wood density profiles be used as proxies for wood anatomical variations? We hypothesize that wood density is driven by the underlying xylem features (especially fibres). Consequently, wood density profiles should help studying the evolution of wood anatomy along the pit-to-bark axis in tropical angiosperm trees. We also

hypothesize that due to the high anatomical variability, this relation will be different from species to species.

Material and Methods

Study sites and samples

Tree increment cores (ø 5.15 mm) were taken at breast height from eight species, one core per species (Table 2-1) in the Democratic Republic of the Congo and stored and dried in paper straws. Those species were selected in order to represent a wide range of wood densities, life strategies and leaf shedding habits. They cover a gradient of wood density varying from 400 to 800 kg/m³ and show various types of anatomical organizations (varying proportions of parenchyma and vessels, large fibres lumina and thin walls to flattened lumina and thick walls; Table 2-2).

Table 2-1 : Summary of the collected species with their family, life strategy and leaf phenology. The average wood density and the diameter of the sampled tree are also given.

Species	Family	WD (kg/m³)	DBH (cm)	Life strategy	Leaf phenology
Anonidium mannii	*Annonaceae*	410.8 (44.3)	26.7	Shade bearer	Evergreen
Canarium schweinfurthii	*Burseraceae*	466.4 (46.8)	31.7	Light demanding	Deciduous
Entandrophragma angolense	*Meliaceae*	630.5 (54.5)	37.2	Light demanding	Deciduous
Milicia excelsa	*Moraceae*	606.8 (53.2)	32	Light demanding	Deciduous
Pycnanthus angolensis	*Myristicaceae*	500.9 (63)	44	Light demanding	Evergreen
Polyalthia suaveolens	*Annonaceae*	815.7 (38.8)	27.5	Light demanding	Evergreen
Staudtia kamerunensis	*Myristicaceae*	784 (45.7)	23.8	Shade bearer	Evergreen
Tetrorchidium didymostemon	*Myristicaceae*	427.5 (38.3)	33.4	Light demanding	Evergreen

WD = wood density. DBH = Diameter at Breast Height. Values between the brackets in the WD column are the standard deviation.

Table 2-2 : Summary of the anatomical diversity of the studied species.

Species	Growth-ring		Vessels			Fibre wall thickness	Parenchyma
	Distinctness	Anatomy	Porosity	Ø (µm)	# / mm²		
Anonidium mannii	2	AC_FD; GC_FD; MP	Diffuse	100 - 200	≤5	68 - 69	78 - 86 - 88
Canarium schweinfurthii	2	GC_FD	Diffuse	100 - >200	<5 - 20	68	75 - 78
Entandrophragma angolense	1 - 2	AC_FW, MP	Diffuse	100 - 200	<5 - 20	68 - 69	78 - 79 - 89
Milicia excelsa	2	GC_FD	Diffuse	≥200	<5 - 20	69	80
Pycnanthus angolensis	2	GC_FD	Diffuse	100 - 200	<5 - 20	68 - 69	75 - 78
Polyalthia suaveolens	1 - 2	AC_FD; MP; DR	Diffuse	100 - 200	<5 - 20	69 - 70	78 - 88
Staudtia kamerunensis	2	AC_FD; MP	Diffuse	100 - 200	5 - 20	70	75 - 78 - 89
Tetrorchidium didymostemon	2	GC_FD	Diffuse	50 - 200	5 - 20	68 - 69	76 - 78

Numbers given in the sections "Distinctness", "Fibre wall thickness" and "Parenchyma" are the categories elaborated by IAWA (IAWA committee, 1989).

Growth-ring boundaries distinctness: 1. Growth ring boundaries distinct; 2. Growth ring boundaries indistinct or absent

Fibre wall thickness: 68. Fibres very thin-walled; 69. Fibres thin- to thick-walled; 70. Fibres very thick-walled

Parenchyma: 75. Axial parenchyma absent or extremely rare; 78. Axial parenchyma scanty paratracheal; 79. Axial parenchyma vasicentric; 80. Axial parenchyma aliform; 86. Axial parenchyma in narrow bands or lines up to three cells wide; 88. Axial parenchyma scalariform; 89. Axial parenchyma in marginal or in seemingly marginal bands. Source: InsideWood (Wheeler et al., 2007).

AC_FD: Abrupt change in fibres diameter; GC_FD: gradual change in fibres diameter; AC_FW: abrupt change in fibre wall thickness; MP: marginal parenchyma; DR: distended rays.

X-ray CT densitometry

Wood cores were placed in paper straws and oven dried for 24 hours before being scanned at 110 µm using the NanoWood CT facility (Dierick *et al.*, 2014), developed in collaboration with XRE (www.XRE.be). The scanned images were reconstructed (GPU GeForce GPX 770 4GB) with the Octopus reconstruction software package ((Dierick *et al.*, 2004; Vlassenbroeck *et al.*, 2007), also licensed by XRE) and further processed (extraction of volumes, tilt correction, tangential correction) through a tailored toolchain (De Mil *et al.*, 2016). X-ray CT wood density profiles result from correcting structure direction of fibre and ring deviations, calibrating with a reference material, and averaging in the tangential and axial direction (Van den Bulcke *et al.*, 2014). The obtained wood density measurements are defined as ovendry wood weight, divided by ovendry volume.

Wood anatomical measurements

Cores were then glued in a wooden sample holder and their transversal surface was sanded with increasingly finer sandpaper (grit 80-1200). The last three centimetres under bark were imaged (StreamMotion, Olympus, Japan) on a scanning stage (SCAN 100 x 100, Märzhäuser Wetzlar, Germany) with a camera (3.2 MP, 2048 x 1536 pixels; UC30, Olympus) mounted on a reflected light microscope (BX60, Olympus, magnification x10). Selecting only the last three centimetres allowed avoiding juvenile wood while already providing a significant amount of data: 250-300 measurements per individual. The final images consisted of several dozens of overlapping images stitched together with the Multiple Image Alignment algorithm (Olympus) and weighing 0.5 GB in average. They were then used to measure the proportions of different tissues (vessels, parenchyma, fibres) and fibres features (the ratio fibre wall/total cell size).

Parenchyma and vessels

As the measurement of vessels, and more particularly parenchyma area is a tedious process, vessels and parenchyma were segmented using the trainable Weka plugin (Arganda-Carreras *et al.*, 2017) in ImageJ (Schneider et al., 2012). The Weka plugin combines a set of machine learning algorithms, to

perform pixel-based segmentations of images. A sub-region was selected as training set and all vessels, parenchyma and fibres were manually labelled. The trained model was then applied on the entire image. A block processing approach was implemented for handling the rather large images and reducing processing speed on the desktop computer (Intel®Core™i5-4570 CPU @ 3.20 GHz 3.20 GHz, 16 GB RAM). The image was therefore subdivided using a user-defined grid, each grid cell was classified separately and all grid cells were stitched after processing resulting in a classification of the entire image. The results are displayed as a probability map per feature (i.e. which is same size as image and where every pixel has a value between 0 and 1, reflecting the probability of being that feature). These probability maps were thresholded with visual assessment for the final delineation of the anatomical features. Misclassifications, such as vessels filled with sawdust, were corrected by indicating vessels manually (Figure 2-1).

Binary maps of vessels and parenchyma were converted to profiles by tangential averaging. The images were also used as masks on the original images prior to measuring the lumina diameters and fibre wall thicknesses (Figure 2-2a).

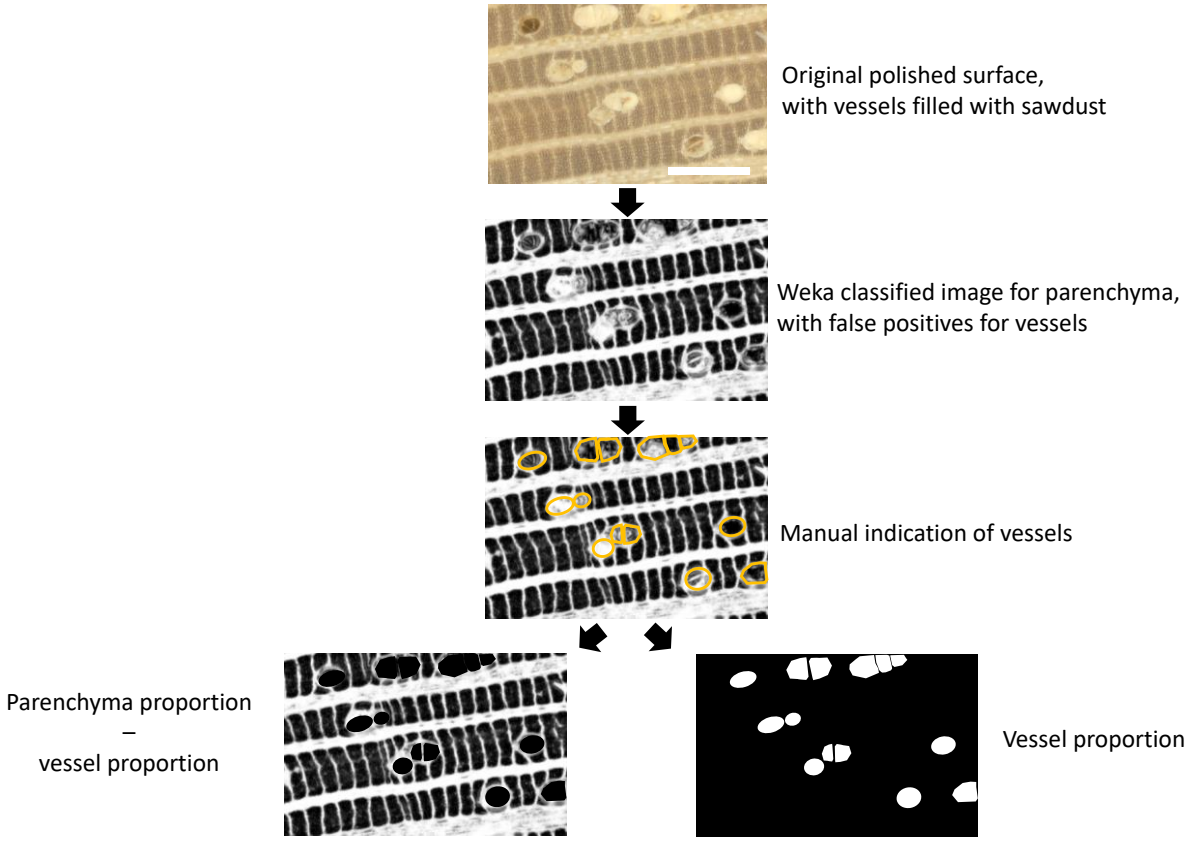


Figure 2-1 : Flowchart for delineating vessels and parenchyma on a segment of a sanded surface with the trainable Weka plugin. The plugin assesses the probability for different portions of the image to be classified as vessel or parenchyma before being manually corrected. Binarized images with only the vessels or the parenchyma were then created. Scale bar 500 µm.

Fibres

Fibre lumina diameter and wall thickness were measured using pattern recognition. First, the images were binarized in order to clearly separate fibre walls from lumina for further steps (Figure 2-2b): light-coloured pixels (corresponding to lumina) were selected with the colour thresholding tool in ImageJ. Adjacent selected pixels formed different shapes that were filtered based on their circularity and their size in order to retain only shapes corresponding to the fibre lumina.

For the detection of individual fibres, we followed the protocol proposed by Kennel *et al.* (2010) consisting of a segmentation, tracking and measurement sequence. Segmentation allows detecting and labelling individual cells based on criteria such as the size, the shape and the surrounding pixel intensity variation (Fiorio & Gustedt, 1996; Wu *et al.*, 2005; Figure 2-2c). Tracking allows connecting different fibres' centroids to form the longest chains in the radial direction by using a nearest neighbours (KNN) algorithm: from the n^{th} centroid, the n^{th+1} was searched within a specified distance

and with a maximum allowed angle of deviation from the radial direction (Figure 2d). Pixel intensity was then measured along the path created by the tracking, with fibres lumina/walls showing pixels with high/low values. The width of fibres lumina and fibre walls was then measured. A user interface was developed to inspect visually the results at each step of the process and to adapt the parameters of the segmentation if required. Implementation of this user interface was based on the Scikit image (Van Der Walt et al., 2014) and Scikit learn (Pedregosa et al., 2012) libraries in Python.

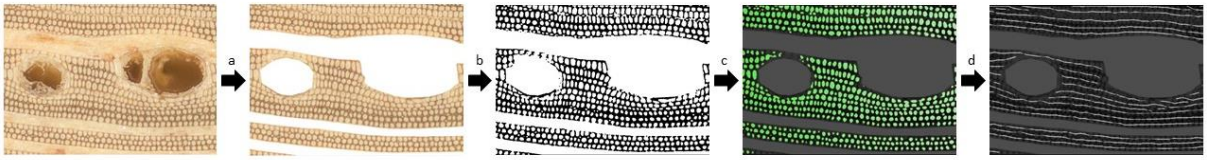


Figure 2-2 : Flowchart of the preparation of a segment of a sanded surface for the measuring of fibre wall thickness and lumina diameter. Step a: the application of a mask (obtained with Weka segmentation plugin in imageJ) on vessels and parenchyma. Step b: binarization of the image in order to separate fibre lumina and walls. Step c: recognition and labelling of individual cells. Step d: tracking and linking of the individual cells. Pixel intensity is then analysed along the created paths and the widths of lumina and fibre walls extracted.

All measurements of the anatomical variables were rescaled to an X-ray CT resolution of 110 µm so that each value of wood density corresponds to a value of fibres/vessels/parenchyma proportion and the ratio of fibre wall/total fibre diameter, further referred to as fibre wall fraction. Fibre proportion, being directly inversely related to the sum of the vessel and parenchyma fraction and thus a linear combination thereof, it was not taken in account for further analyses and model construction.

Statistical analysis

For the analysis of the local relationship between wood density and wood anatomy, we subdivided the density and anatomical measurements profiles into ten sections of 30 data points and constructed a linear model with parenchyma, vessels and the fibre wall fractions as explicative variables. All anatomical measurements data was standardized per species prior to the statistical study in order to assess the relative importance of the three variables.

For the overall relationship between wood density and anatomy, we used the linear mixed framework with random intercepts and slopes to investigate the potential of predicting wood density using the

proportion of vessels, parenchyma and fibre wall fraction as fixed effects and species as random effects (Eq. 1). This model allows assessing the importance of the species effect in the relationship between wood density and wood anatomy as it accounts for the species effect on the relationship between the predictor variables and the response.

$$WD_{i,j} = \beta_{0,k} + \beta_{1,k} x_{i,j,k} + \alpha_{0,j,k} + \alpha_{1,j,k} \varepsilon_{i,j,k} + \varepsilon_{i,j} \quad \text{(Eq. 1)}$$

Where WD(i) and WD(i,j) are i-th measure of wood density of species j, beta(0,k) is the intercept and beta(1,k) is the slope for the k-th variable regardless of species, alpha(0,j,k) is the deviation from the global intercept and alpha(1,j,k) is the deviation from the global slope for the k-th variable of species j.

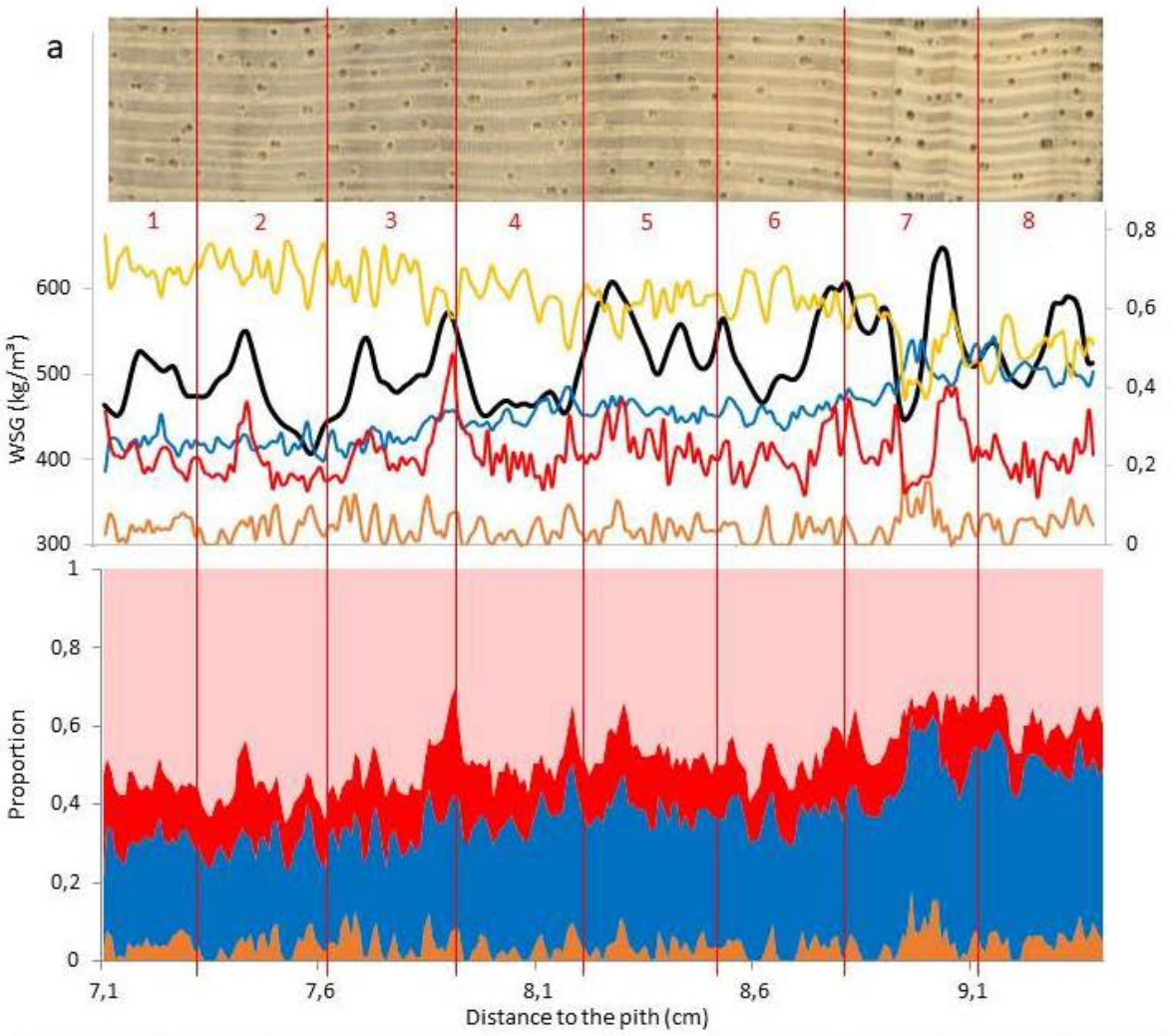
Results

Average wood density values range from 510 kg/m³ to 794 kg/m³. On average, fibres show the highest proportion, followed by parenchyma and vessels. All relative proportions of anatomical features per species are presented in Table 2-3. Fibre wall fraction of the fibre proportion part, varies between 22 and 49 %.

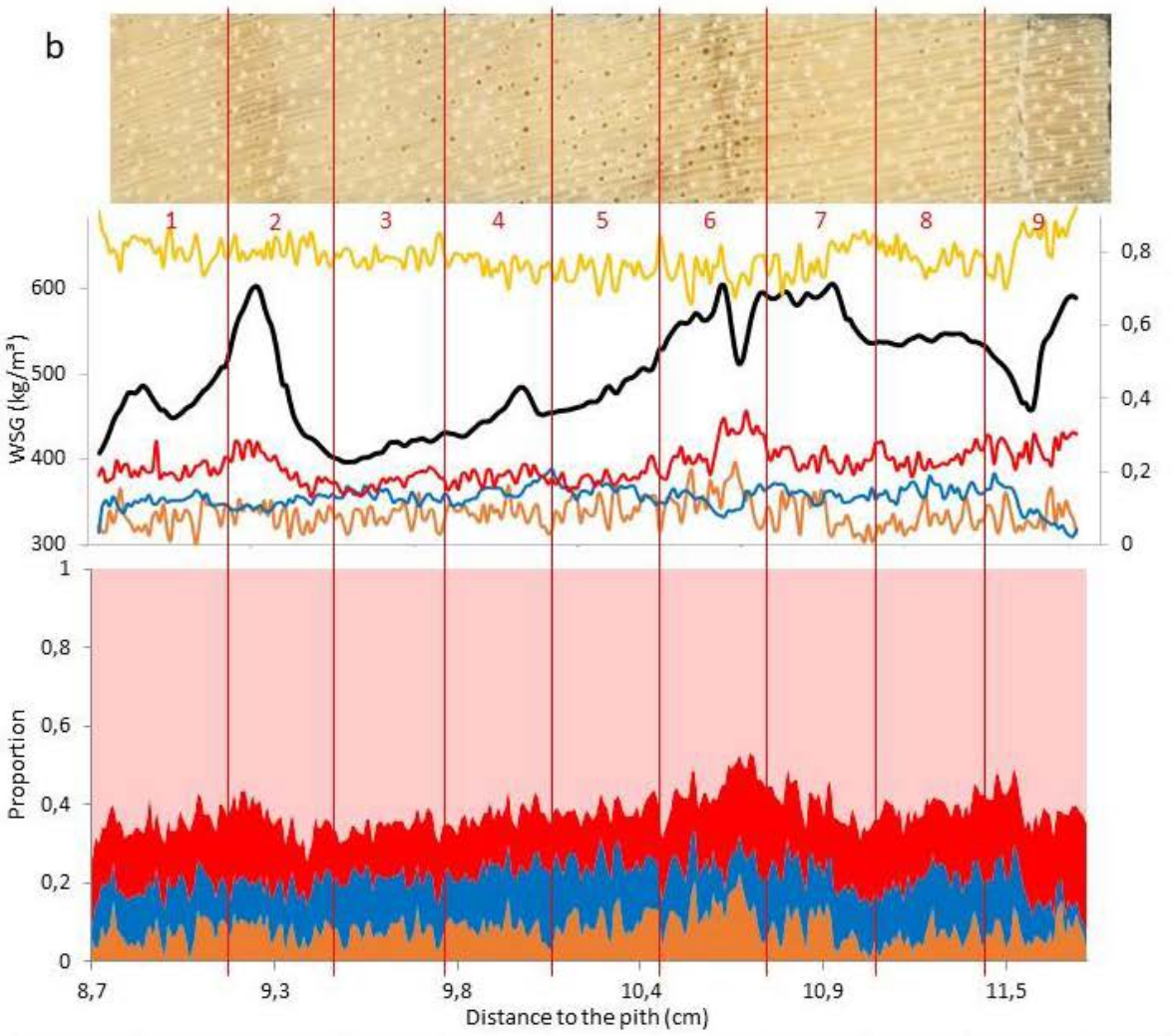
Table 2-3 : Wood density (WD, kg/m³), parenchyma proportion, vessel proportion, fibre proportion, as well as fibre wall fraction (defined as the fibre wall width divided by the sum of fibre wall and lumen diameter). Standard deviation is given between brackets.

Species	WD (kg/m³)	Parenchyma (%)	Vessels (%)	Fibres (%)	Fibre wall fraction (%)
A. mannii	513.15 (48.77)	0.34 (0.07)	0.04 (0.03)	0.62 (0.09)	0.23 (0.06)
C. schweinfurthii	500.91 (60.75)	0.13 (0.03)	0.09 (0.04)	0.78 (0.04)	0.22 (0.04)
E. angolense	651.89 (47.61)	0.23 (0.07)	0.08 (0.04)	0.69 (0.08)	0.35 (0.06)
M. excelsa	571.17 (57.71)	0.28 (0.14)	0.06 (0.04)	0.66 (0.16)	0.25 (0.06)
P. angolensis	570.79 (36.30)	0.23 (0.03)	0.07 (0.04)	0.7 (0.04)	0.33 (0.03)
P. suaveolens	794.36 (25.82)	0.42 (0.03)	0.12 (0.04)	0.46 (0.04)	0.49 (0.06)
S. kamerunensis	768.67 (26.51)	0.31 (0.03)	0.05 (0.02)	0.64 (0.03)	0.35 (0.05)
T. didymostemon	510.30 (30.72)	0.19 (0.08)	0.13 (0.05)	0.67 (0.09)	0.25 (0.05)

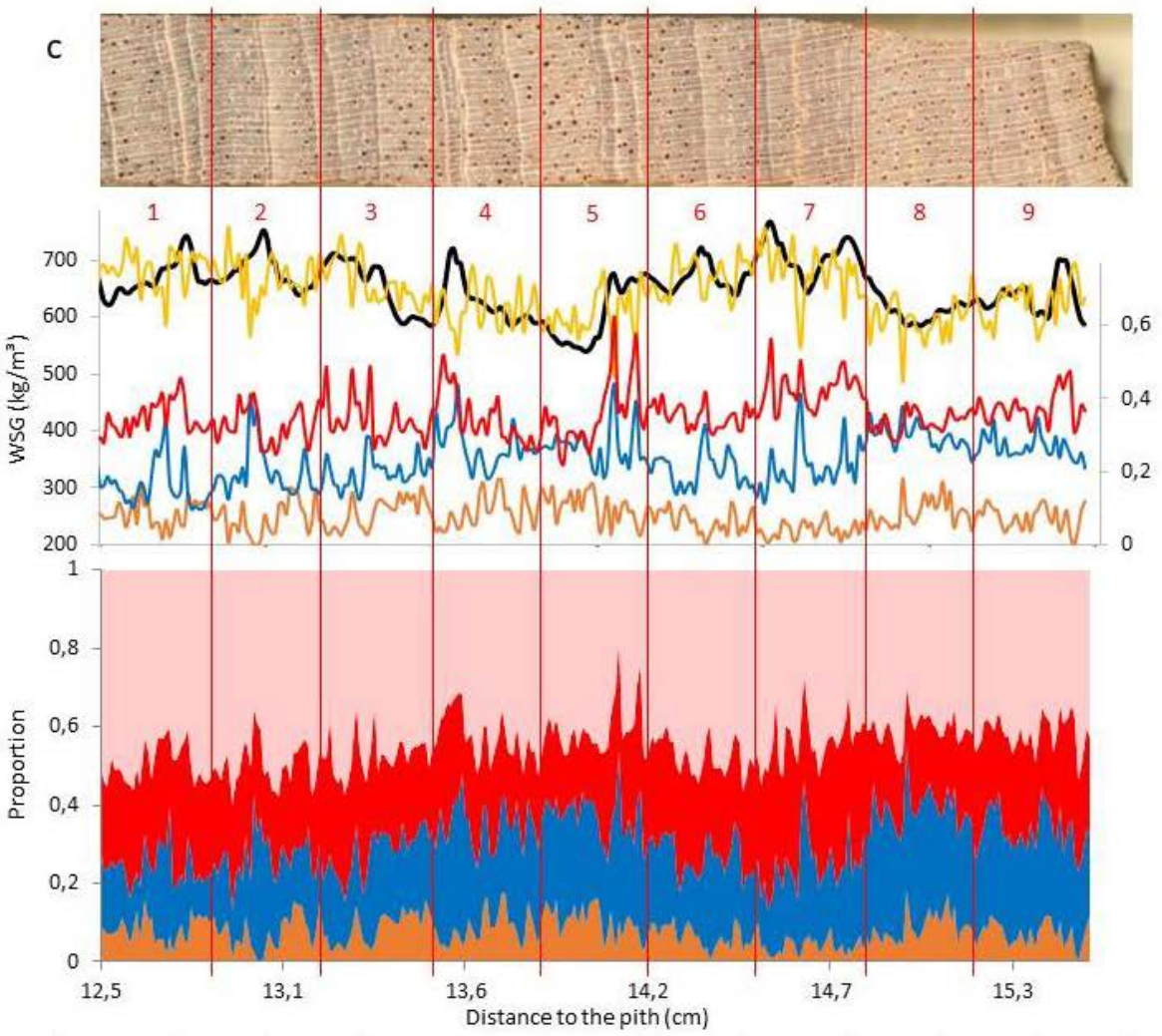
All species have varying radial patterns of wood density. Some species show small, high-frequency density variations along the profile (Figure 2-3 f, g), while other species show large, low-frequency variations (Figure 2-3b), seen as narrow and wide growth zones respectively. Visual assessment of the top panel of Figure 2-3 shows that measured anatomical features are linked with the variability in wood density. The most important driver of wood density variability, regardless of the species, appears to be the fibre wall fraction (i.e. the ratio fibre wall thickness/total fibre size): higher fibre wall fractions increase wood density (Figure 2-3). Moreover, for some species, the fibres proportion is also linked with wood density (Figure 2-3 c, d, h). The relationship between wood density and parenchyma or vessels is less straightforward: while parenchyma is correlated with wood density either positively (Figure 2-3a), negatively (Figure 2-3 c, d, h) or only marginally (Figure 2-3 b, e, f, g), vessel correlation with wood density is, in most cases, the weakest of all the anatomical features. The local relationship between wood density and wood anatomy reveals varying trends (Figure 2-3 lower panels). While the influence of fibres remains positive throughout the profile, the influence of parenchyma and vessels can vary substantially from one sub-section of the core to the other: parenchyma can be both positively and negatively correlated to wood density within one individual (*e.g.* Figure 2-3c) and vessels, although not significant overall, can play an important role locally (*e.g.* Figure 2-3a).



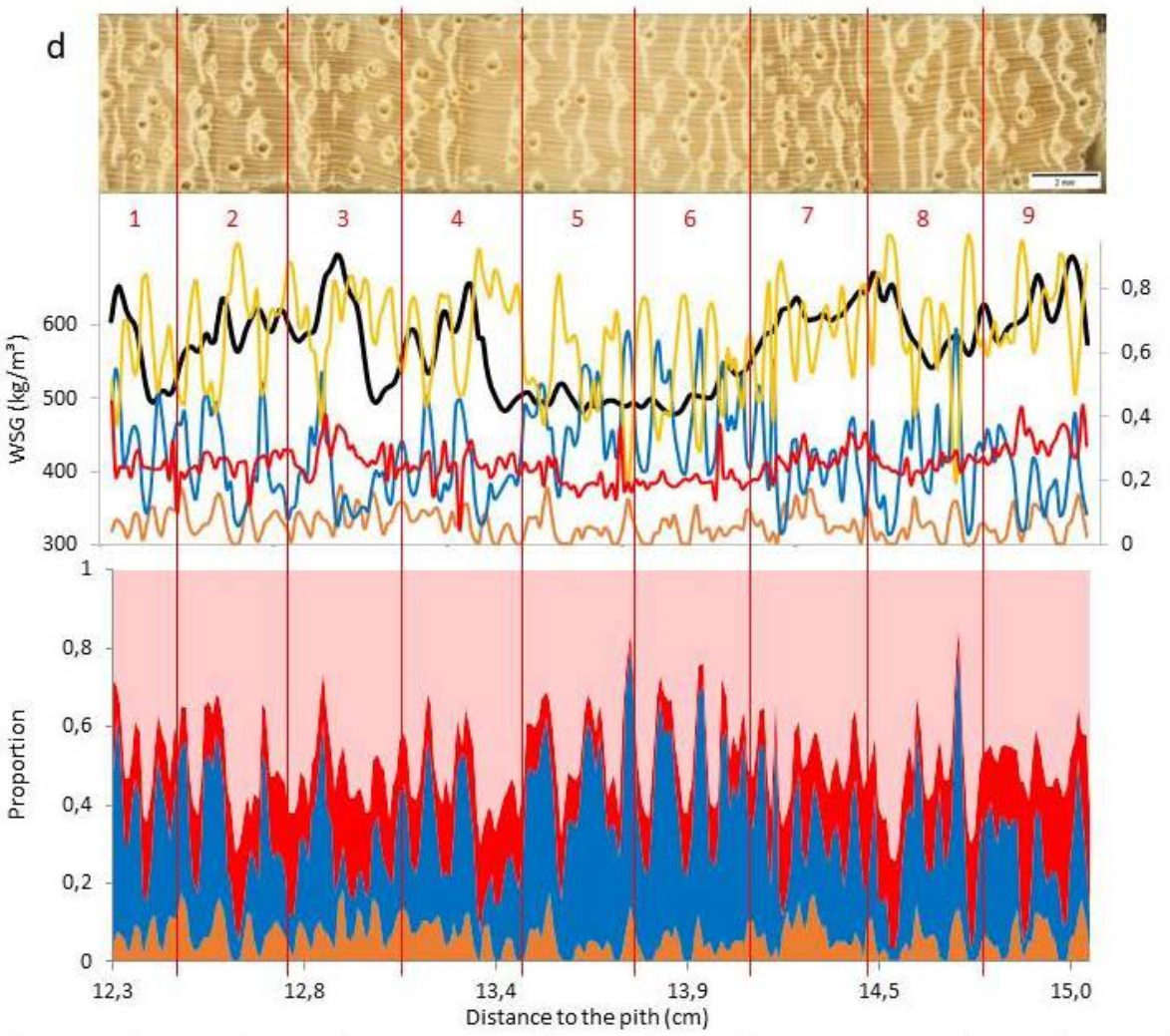
	1	2	3	4	5	6	7	8	Total
Inter-cept	505.4 ***	512.7 ***	500.1 ***	475.6 ***	540.3 ***	526.7 ***	580.9 ***	559.5 ***	513.1 ***
Paren-chyma	13.8	20.4	5	-2.5	49.6 *	36.3	-31.4 .	-20.9	17.2 ***
Vessels	-1.1	-11.9 *	-0.7	-2.1	3.3	2.8	2.3	15.2 .	-2
Fibre Wall	-9.7	37.4 ***	28.3 ***	18.1 ***	16.1 *	23.7 **	23.1 **	2.8	25.1 ***



	1	2	3	4	5	6	7	8	9	Total
Inter-cept	470.3 ***	477.5 ***	427.4 ***	449.2 ***	503.4 ***	550.3 ***	582.5 ***	541.9 ***	498.8 ***	500.9 ***
Paren-chyma	1.1	-51.8 ***	-4.4	15.2 **	-7.3	-8.6	9 .	0.9	-7.5 .	4.1
Vessels	-0.5	-3.4	-0.8	5.5	4.9	-3.5	5.5 *	1.8 .	11.8	3.7
Fibre Wall	9.2	46.8 ***	15.6 ***	8.5	27.5 **	4.4	2.3	-0.4	20.4 *	43.2 ***



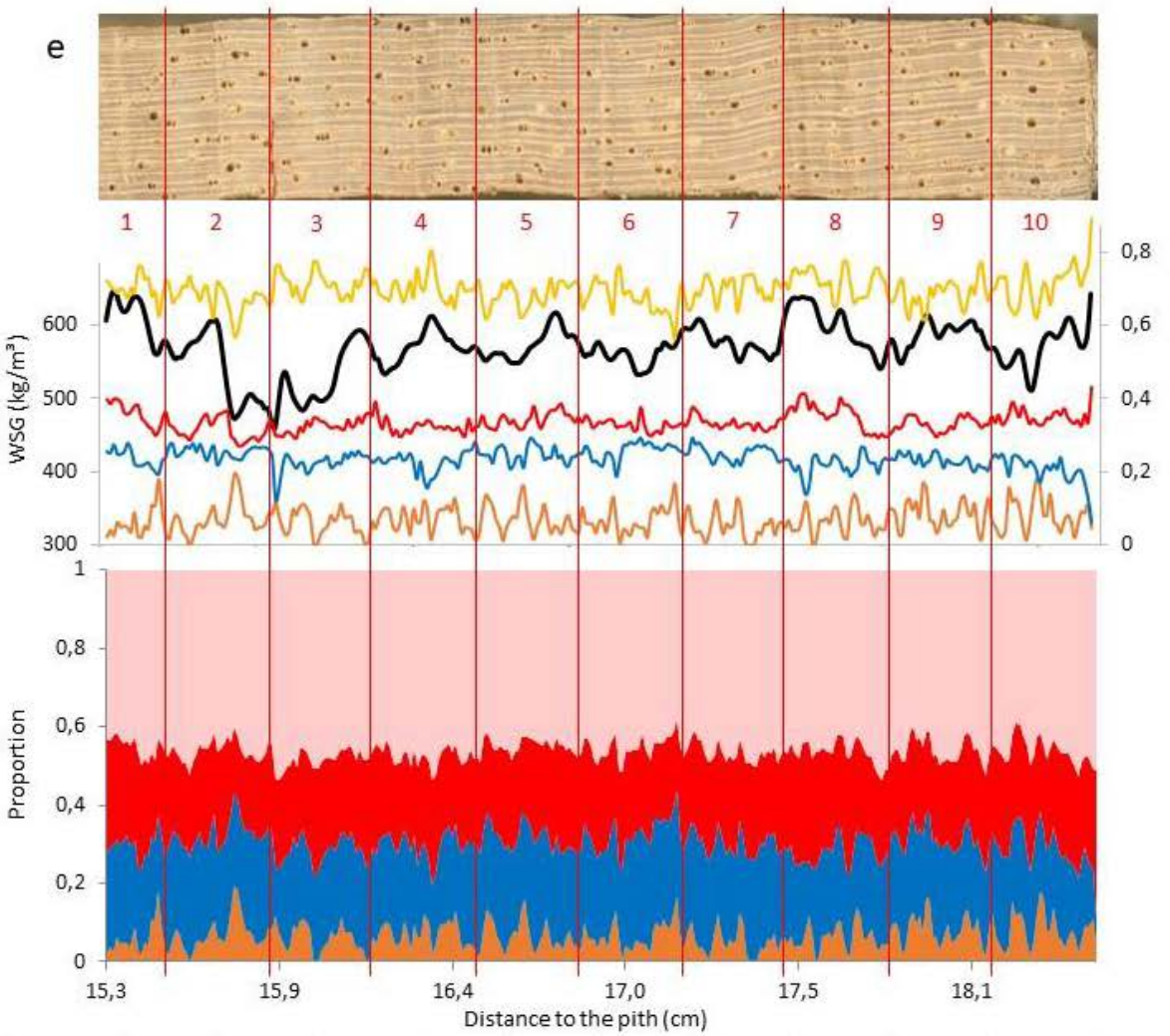
	1	2	3	4	5	6	7	8	9	Total
Inter-cept	672.6 ***	680.9 ***	656.4 ***	618.1 ***	619.1 ***	667.1 ***	664.1 ***	609.7 ***	622 ***	651.9 ***
Paren-chyma	2.5	12.3 *	-18.2	16.3 *	-17.8 *	2.9	-11.5 *	1.5	6.8	-16.6 ***
Vessels	9.9	-7.9 .	-27.3 ***	3.4	-13.7 *	-14.4 *	-18.1 *	-7.4 *	-19.7 ***	-19.5
Fibre Wall	21.5 *	-8.3	8.7	14.6 *	26.9 ***	3.9	21.3 **	-12.7	27.6 ***	16.9 ***



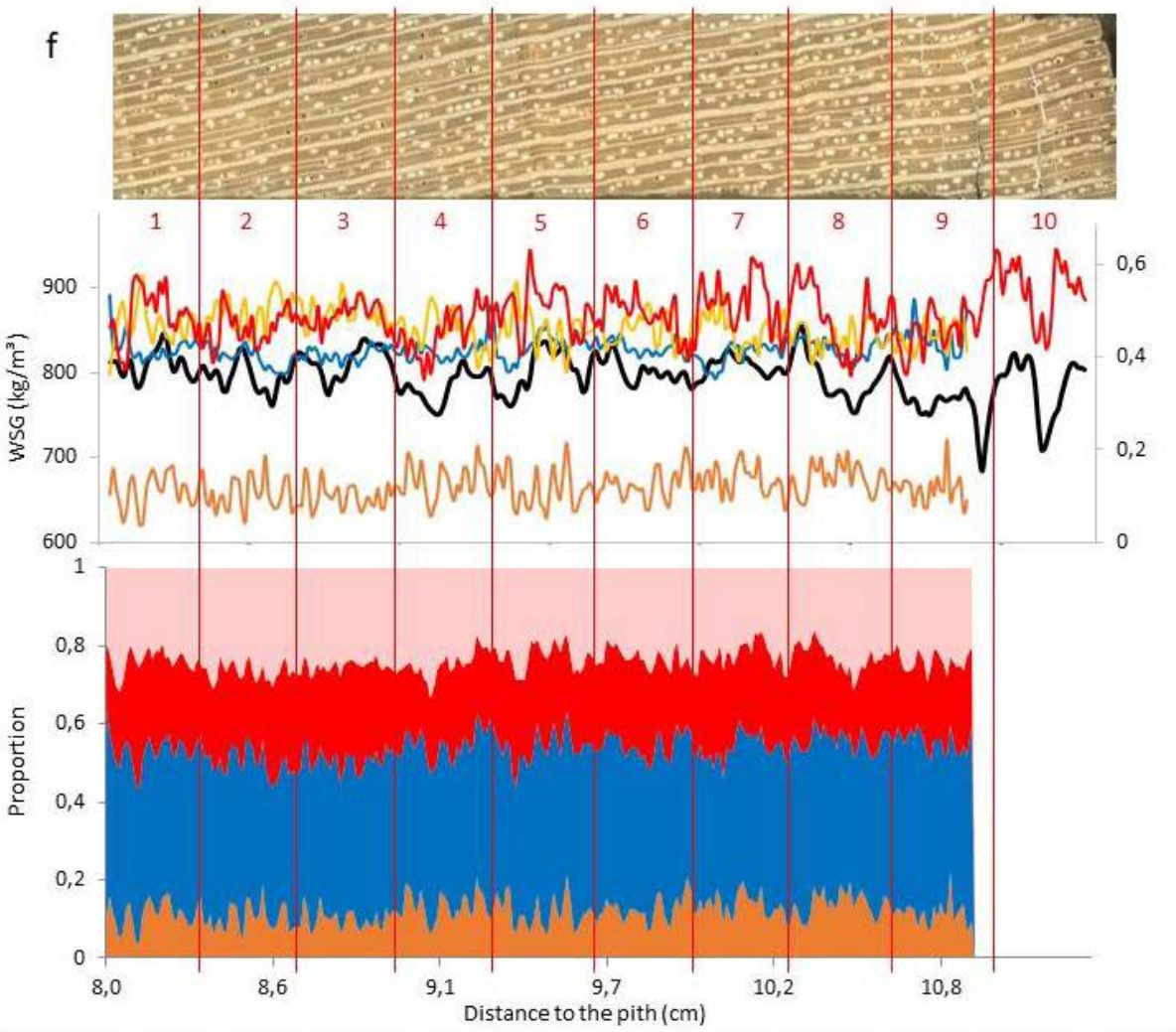
	1	2	3	4	5	6	7	8	9	Total
Inter-cept	556.9 ***	589.9 ***	574.5 ***	565.3 ***	501.9 ***	511.9 ***	603.7 ***	594.4 ***	612.5 ***	571.1 ***
Paren-chyma	26.2	-3.1	0.4	1.3	-5.9 **	0.8	-2.9	-11.4	10	-12.1 ***
Vessels	-23.4	1.1	-6.5	21.7 .	-1.2	1.8	2.1	2.5	17.6 **	8.2 **
Fibre Wall	-0.1	17.5 **	39.8 ***	5.9	1	7.8	21.8 ***	12.1	15.1	27.4 ***

Wood density Fibre proportion Parenchyma proportion Vessel proportion Wall/Fibre diametre

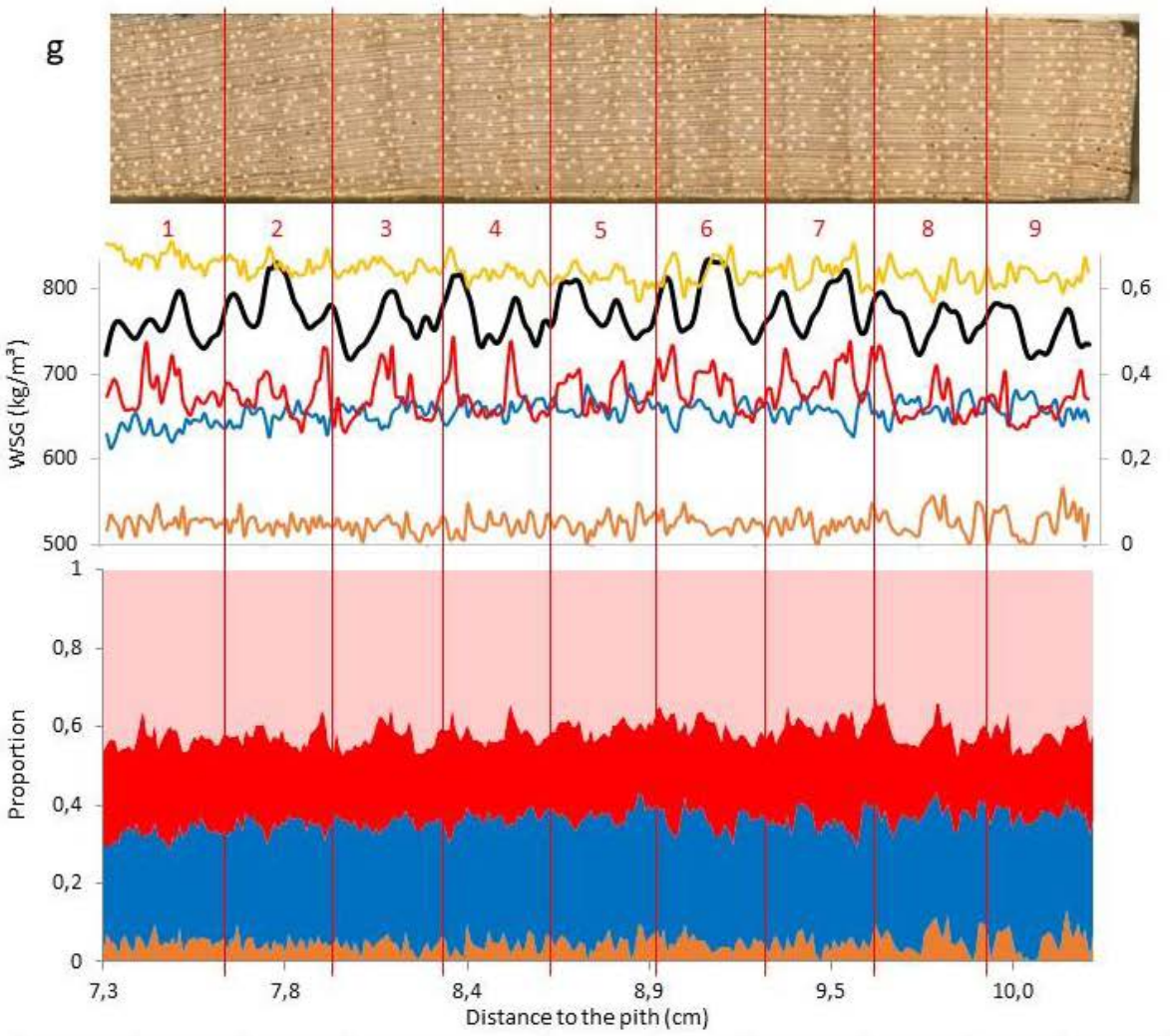
Fibre walls Fibre lumina Parenchyma Vessels



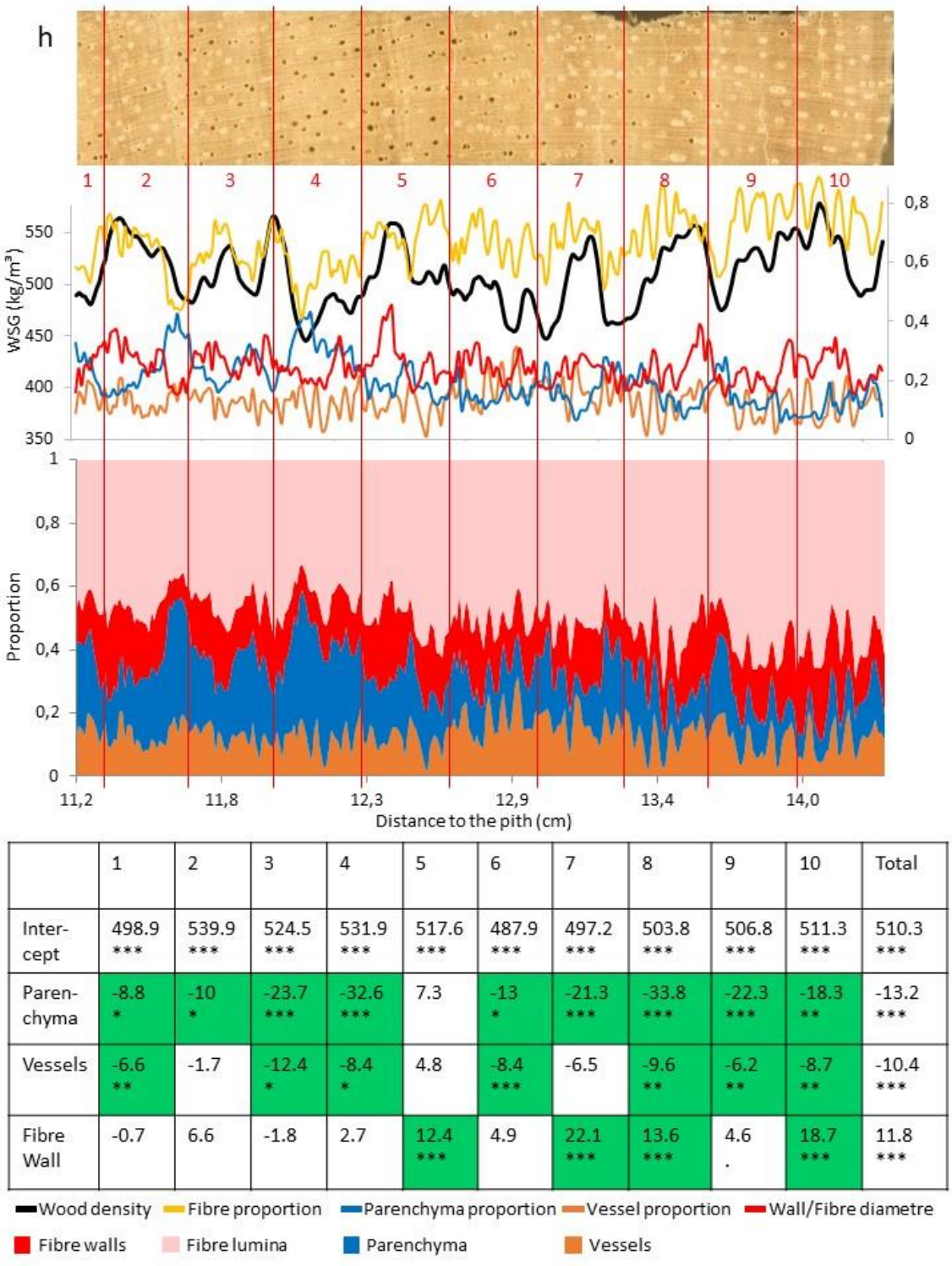
	1	2	3	4	5	6	7	8	9	10	Total
Inter-cept	599.9 ***	565.2 ***	530.8 ***	563.8 ***	570.3 ***	566.7 ***	576.9 ***	584.9 ***	585.1 ***	557.8 ***	570.8 ***
Paren-chyma	-4.3	-4.7	19.3 *	-16.8 ***	-2.7	-7.1 *	3.3	0.8	-10.5 .	-12.8 ***	-1.9
Vessels	-11.7 *	-14.3 *	-9.9	-3.9	-2.9	3.4	-2.1	-5.4	1.8	-7.7 *	-4.7 *
Fibre Wall	14.5 **	22.8 ***	2.6	-5.3	17.6 ***	2.5	16.7 ***	20.3 ***	-1.6	4.3	19.7 ***



	1	2	3	4	5	6	7	8	9	10	Total
Inter-cept	810.7 ***	800.5 ***	818.9 ***	788.7 ***	791.8 ***	800.6 ***	804.1 ***	794.1 ***	772 ***	/	798.4 ***
Paren-chyma	1.6	9.6 **	13.8 *	6.1 .	16.7 ***	10.4 *	1.3	12.6 *	3.6	/	3.3 **
Vessels	7.8 *	2.2	-0.4	4.2	3.3	0.4	3.3	-1.3	5.4	/	-0.8
Fibre Wall	1.8	0.6	17.6 *	9.6 **	6.9 .	1	1.4	13.4 ***	5.86	/	10.3 ***



	1	2	3	4	5	6	7	8	9	Total
Inter-cept	736.1 ***	776.8 ***	764.9 ***	769.7 ***	783.7 ***	777.6 ***	770.9 ***	769.3 ***	749.5 ***	768.7 ***
Paren-chyma	-17.1 **	-16.6 *	8.2	1.3	-16.7 **	-0.4	-9.1 *	-8.9 *	4.8	-0.8
Vessels	-6.4	-12.8 .	10 .	-1	-11.3 **	-14.1 *	-6.1 .	-3.1	7.2	-2.6 .
Fibre Wall	0.3	-6.7	13.1 ***	15.8 **	6.6	21.5 ***	13 **	10.6 **	-0.02	14.2 ***



	1	2	3	4	5	6	7	8	9	10	Total
Inter-cept	498.9 ***	539.9 ***	524.5 ***	531.9 ***	517.6 ***	487.9 ***	497.2 ***	503.8 ***	506.8 ***	511.3 ***	510.3 ***
Paren-chyma	-8.8 *	-10 *	-23.7 ***	-32.6 ***	7.3	-13 *	-21.3 ***	-33.8 ***	-22.3 ***	-18.3 **	-13.2 ***
Vessels	-6.6 **	-1.7	-12.4 *	-8.4 *	4.8	-8.4 ***	-6.5	-9.6 **	-6.2 **	-8.7 **	-10.4 ***
Fibre Wall	-0.7	6.6	-1.8	2.7	12.4 ***	4.9	22.1 ***	13.6 ***	4.6 .	18.7 ***	11.8 ***

Figure 2-3: Sanded wood core sections plotted along the corresponding density profiles (black line, left Y-axis) and the measurements of anatomical features (coloured lines, right Y-axis) in the upper panel. The central panel represents the evolution of the proportions of the fibre walls (red), fibre lumina (pink), parenchyma (blue) and vessels (orange). The lower panel represents the moving window (window width = 30 density values) regression coefficients between WD and parenchyma, vessels and fibre wall fraction. Windows with significant coefficients are emphasized in green. a) Anonidium mannii, b) Canarium schweinfurthii, c) Entandrophragma angolense, d) Milicia excelsa, e) Pycnanthus angolensis, f) Polyalthia suaveolens, g) Staudtia kamerunensis, h) Tetrorchidium didymostemon.

These trends are confirmed by the mixed model (Table 2-4). Overall, fibre wall fraction is positively correlated with wood density while vessels and parenchyma slightly decrease it. Fibre wall fraction impacts density the most, followed respectively by vessel and parenchyma proportions. The model also shows the importance of the species effect on the relationship between wood density and wood anatomy: 27 % of wood density variability is influenced by species.

Table 2-4: Coefficients from Gaussian linear mixed model (random intercept + slope) predicting the wood density from the anatomical variables for the eight tropical species investigated. Coefficient estimates are provided for the fixed effects (at 95% of confidence interval).

Fixed effects	
Intercept	490.92
Parenchyma proportion	-24.39
Vessels proportion	-94.22
Fibre wall fraction	453.67
% of variance	65 %
Random effects	Estimated contribution to total variance
Species	4 %
Species|parenchyma	4 %
Species|vessels	6 %
Species|fibre walls	13 %
Residuals	8 %

Moreover, the trends observed at the species level can be completely different from the overall average trend (Figure 2-4). While the slopes of the regression lines between wood density and the fibre wall fraction are variable between species but remain all positive (Figure 4a), the effects of parenchyma or vessels can heavily vary between species (Figure 2-4b, c).

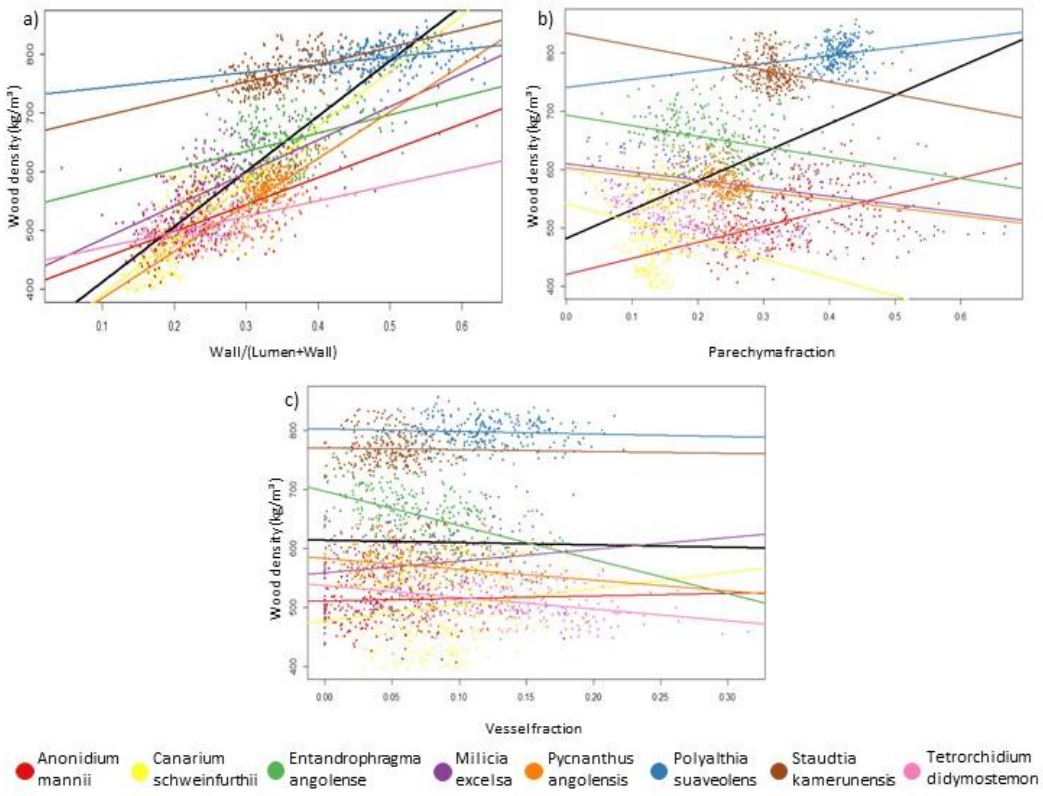


Figure 2-4 : Relationship between wood density and different anatomical features for all studied species (coloured lines) and overall (black line). Each point represents a density value and the correspondent value of a) fibre wall fraction, b) parenchyma proportion and c) vessel proportion.

Discussion

Wood anatomy and wood density relationship

The differences in wood density between species are driven by their anatomy. The central panels of Figure 2-3 show that the denser species show the biggest proportions of fibre walls and parenchyma while the lowest densities are mostly caused by the highest proportions of fibre lumina. Vessels don't significantly influence wood density overall (Figure 2-4c). Although only one individual per species was used in this study, differences in wood anatomy observed between species were larger than between conspecific individuals observed in the RMCA collection. The nature, shape and grouping of wood cells are constant for a given species but since wood is a material of biological origin, its anatomical structure varies between conspecific individuals. However, since only one individual per species was used in this study, little can be said about the inter-individual variability of the wood density – wood

anatomy relationship. Therefore, we tackle the the observed variability of the wood density – wood anatomy from a species point of view, rather than individuals.

The positive influence of fibre walls on wood density was already described by previous studies by Roque & Tomazelo-Filho (2007), Ziemińska *et al.* (2013) or Dadzie *et al.* (2016). Fibres proportion and the fraction of walls within the fibre determine the area occupied by walls and by lumina which exerts the strongest influence on wood density. However, locally, fibres don't always significantly influence wood density (Figure 2-3). Usually, at these sections the fibre wall fraction variation is only marginal and other tissues (parenchyma and, less frequently, vessels) drive wood density variations. When the low variation of fibre wall fraction concurs with a constant proportion of vessels and parenchyma, the density variation will be minimal as well.

There is a large variability in the relationship between the parenchyma proportion and wood density: although we reported a positive overall relationship between parenchyma and wood density, the parenchyma is negatively correlated with wood density in most species (Figure 2-4b), concurring thus with the findings of Martínez-Cabrera *et al.* (2009). Although, for technical reasons, parenchyma was simplistically considered as a tissue with uniform properties and influence on wood density, it is a complex tissue with different cell types and dimensions: rays and axial parenchyma have different biological functions that influence their size, chemical composition and density (Spicer, 2014; Morris *et al.*, 2016). The observed inter-species variability of the parenchyma effect on wood density may thus be driven by differences in parenchyma cells between species (chemical extractives, crystals, cell walls thickness). Moreover, species-specific interactions between parenchyma and other xylem features can also occur: parenchyma bands can be associated with vessels (*i.e. Milicia excelsa*, Figure 2-3d), which potentially mixes the effects of both features and makes challenging to measure the influence on wood density of parenchyma alone.

The link between wood density and vessel proportion is also variable. Although vessels represent "void volume" (Lachenbruch & Mcculloh, 2014) and can locally significantly impact wood density (Figure 2-

3), they have only a limited influence overall (Figure 4c, Poorter *et al.*, 2010). Moreover, vessels "void" negative effect on wood density can be counteracted by other anatomical factors such as associated parenchyma or fibres of reduced size. Our results are contrasting with papers that dedicate intra-annual density fluctuations to vessel changes and the direct link with hydraulic properties (Battipaglia *et al.*, 2016).

Our results stress the importance of studying the species-specific wood anatomy - wood density relationship at high spatial resolution. Different anatomical features proportions vary within different ranges and their effect on wood density varies in function of the studied species and the position within the growth ring. Such high-resolution variability is difficult to capture if studying only averaged values of anatomical measurements over large sections of wood so that several trends (*e.g*. the vessel - density relationship) might be missed out.

Density profiles as a proxy for wood anatomical variations

Brienen *et al.* (2016) suggested that high-resolution density profiles could assist in tropical tree-ring detection and analysis. We showed that wood density is closely related to fibres wall variation, theoretically allowing to detect sections with flattened fibres with densitometry. Species such as *A. mannii* or *S.kamerunensis* allow that type of approach (Figure 2-3 a,g). However, with increasingly gradual variations (*C. schweinfurthii* – Figure 2-3b, *T. didymostemon* – Figure 2-3h), density peaks, as growth-ring boundaries, become less clear. Moreover, although several tropical species show ring boundaries defined by density fluctuations due to the fibre wall thickness variations (Worbes, 2002; De Ridder *et al.*, 2011), we show that other types of tissue also influence wood density and that other density variations are due to features other than fibres (*e.g.* M. excelsa – Figure 2-3d). This additional source to the variability of the density profile makes fibre-driven density variations difficult to detect among all the others (Worbes, 1995). Furthermore, our results highlight the complexity of the relationship of wood density with parenchyma and vessels: it can significantly vary between species

but also throughout a pith-to-bark profile. So far, it appears impossible to detect rings delimited by parenchyma bands or vessels as defined in Worbes (2002).

An increasing number of studies stresses the importance of analysing intra-annual fluctuations in tree growth studies (Fonti *et al.*, 2010; Battipaglia *et al.*, 2016). Studying continuous variation of anatomical features instead of focusing only on ring boundaries allows considering density profiles in climate studies. To the best of our knowledge, our study is the first to provide simultaneous measurements of three xylem tissues along a radial gradient. We showed that their relationship with wood density is species-dependent. To further disentangle the wood density – wood anatomy relationship we recommend more thorough species-specific studies of wood density. When including cambial phenology (De Mil *et al.*, 2017), climatic events could be linked to annual or intra-annual density fluctuations. Also extractives, crystals and cell content could be of importance.

The ability to use wood density as a proxy for wood anatomy would represent a significant advance in tree studies since sample preparation, image acquisition and cell measuring are tedious and time consuming. On the contrary, modern techniques allow a fast acquisition of high-resolution wood density profiles (De Mil *et al.*, 2016) thus increasing the number of studied trees and the geographic coverage of the studies. A better knowledge of the wood density - wood anatomy relationship would enable us using these large density databases in wider frameworks.

Reflections on methodology

Manually measuring xylem features is a time-consuming process (von Arx & Carrer, 2014). Automated measurements are needed yet hampered by the diversity of cell types, shapes, sizes, especially in tropical hardwoods. Different techniques and softwares allow analysing different anatomical features: *e.g.* ImageJ allows routinely analyse vessels and ROXAS allows studying tracheids of softwoods (von Arx & Carrer, 2014). Based on previous studies emphasizing the influence of fibres on wood density (Zieminska *et al.*, 2013), we developed a technique that allows further exploring the wood anatomy – wood density relationship by detecting and measuring the fibres features of the sample.

The success of these techniques relies on the quality of the image and, therefore, of the sample preparation. More particularly, the contrast between fibre walls and lumina and vessels lumina and a uniform coloration of lumina are vital for an automated detection of individual anatomical features. A trade-off between the quality of the sample and its size must be made: thin sections usually offer a good contrast between different anatomical features but on a little area while sanded surfaces allow working on significantly larger number of samples (Hoadley, 1990) but with reduced contrast due to dust filling fibres and vessels lumina (Gartner & Nievergelt, 2010). We also recommend further efforts in the improvement of surface sanding techniques, especially directed towards the increasing of the contrast between anatomical features and the uniformisation of lumina coloration.

Conclusion

Continuous wood anatomical features variation is increasingly being used to reveal tropical tree growth in the past and present. Manually measuring xylem features is tedious and time consuming while automated measurements still require important supervision. In this study we proposed X-ray microdensitometry profiles as proxies for wood anatomical features such as vessels, parenchyma and fibres. The decomposition of the density profile in its anatomical features would allow a correct interpretation of density fluctuations and provide a fast and non-destructive technique for analysing intra-annual variations in tropical trees.

We showed that wood density variation is correlated with the variability of vessels area, parenchyma area and mostly fibres anatomy variation and that microdensitometry is able to map intra-annual fluctuations. Fibre wall fraction has the largest effect on wood density, whereas vessels and parenchyma affect wood density more locally, to a lesser and more varying way. These relations are, however, species-dependent and require a more thorough analysis for a set of important species (analysing a greater number of conspecific individuals, on the entire profile from the pith to the bark, subdividing parenchyma in rays and axial parenchyma).

Acknowledgments

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Chapter 3. Study of the influence of the precipitation seasonality on wood density variability

Chapter 3: Study of the influence of the precipitation seasonality on wood density variability

Preface Chapter 3

In the previous chapter we showed some general trends in the wood density – wood anatomy relationship. A close relationship between fibres anatomy and wood density was highlighted. The variation in fibres diameter is also among the most common markers of variations of cambial activity. Although more thorough research is needed, we believe that wood density profiles can be used as proxies to study wood anatomy variations and, ultimately, wood growth.

In the present chapter we aim to explore the opportunities opened by the previous study. Although the current state of our knowledge doesn't allow to link specific climatic events to specific portions of density profiles, we think that some trends in wood growth can be studied. In particular, we use the opportunity to quantify the wood density signal with different metrics in order to offer means of objective comparison between different trees or groups of trees.

Chapter 3: Study of the influence of the precipitation seasonality on wood density variability

Title

Wood anatomy variability under contrasted precipitation seasonality of common deciduous and evergreen species from central African forests.

Authors

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Authors contribution

- Paper conception: Tarelkin, Hufkens, Van den Bulcke, Bastin, Beeckman, De Cannière
- Samples collection and preparation: Tarelkin, Hufkens, Van den Bulcke
- Analyses: Tarelkin, Hahn, Debeir, Van den Bulcke
- Major review: Tarelkin, De Cannière, Van den Bulcke, Hufkens, Bastin
- Minor review: all the co-authors

Submitted in *Trees* in March 2018

Abstract

Tropical forests face increasing pressures due to climate change and yet, the response of trees to varying climate conditions remains poorly understood. In the present study, we aim to fill some knowledge gaps by comparing the leaf phenology and the pith-to-bark wood anatomical variability of 13 common tree species of the Democratic Republic of the Congo among three sites presenting contrasted rainfall regimes. We measured pith-to-bark density profiles on which we applied wavelets analyses to extract three descriptors, which we further used as proxies to describe and compare wood anatomical variability. They describe the periodicity, regularity and the amplitude of variations of the anatomical features and, therefore, reflect tree growth patterns. Our results show that evergreen species tend to have significantly higher anatomical variability where rainfall seasonality is more pronounced. Deciduous species, in spite of shedding leaves for longer periods in drier sites, did not show significant differences in their anatomical variability. The analyses of density profiles and phenology records suggest that the seasonality of precipitation influences both leaf phenology and cambial activity. The high intra-site variability in phenology and anatomy within species suggests that site-related micro-environmental conditions also influence cambial activity.

Key message: Wood density profiles revealed significant differences in wood formation along a precipitation gradient in the Congo Basin. The adaptation of trees to different environmental conditions varies depending on leaf phenology properties.

Keywords: wood density, wood anatomy, leaf phenology, wavelet analysis, tropical tree growth, environmental conditions

Introduction

Tropical forests cover about 7% of the terrestrial surface and store about 25% of its carbon (Bonan *et al.* 2008; Pan *et al.* 2011). They are key players in the global carbon cycle (Bonan 2008; van der Werf *et al.* 2009; Pan *et al.* 2011; Sexton *et al.* 2016) and provide short- and long-term services to the society. Tropical forests, however, are increasingly threatened by anthropogenic pressure (FAO 2016) and global warming (James *et al.* 2013). Extreme climatic events and the prolonged dry seasons (IPCC 2013) are expected to aggravate tree mortality and to affect tree growth (Allen *et al.* 2010; Phillips *et al.* 2010; Greenwood *et al.* 2017). Although substantial shifts in growth and biomass allocation were observed in the tropical forests (Chave *et al.* 2008; Lewis *et al.* 2009; Dong *et al.* 2012), these processes have yet to be understood in the framework of climate change. A better understanding of the influence of the climate change on forest dynamics is therefore essential to predict the future of tropical forests (Rozendaal and Zuidema 2011; Pucha-Cofrep 2015) and to elaborate adapted forest management techniques (Scheffer *et al.* 2001; Phillips *et al.* 2010).

Current knowledge on tree sensitivity to climate change is mainly based on experimental studies of leaf-level physiological responses, but the exact influence of climate on the growth of woody biomass remains largely unknown (Zuidema *et al.* 2013). Moreover, our understanding of the influence of climate seasonality on leaf phenology in the tropics is limited (Moreau 2016). Remote sensing studies, which cover large geographic extents at high temporal resolution, show promising results in research on vegetation responses to seasonal climatic changes (Viennois et al. 2013; Philippon *et al.* 2016). Yet, persistent artefacts due to acquisition conditions induce errors in the estimations of the vegetation response to climate variability (Morton *et al.* 2014). Moreover, species-specific monitoring is hampered by the high canopy diversity (Baldeck *et al.* 2015). Additional field data to support remote sensing data and refine the estimates of forest productivity and carbon storage are thus needed (Babst *et al*. 2014; Moreau 2016).

It is well known that seasonal variation of environmental conditions such as precipitation or solar irradiance drives leaf shedding (Couralet *et al.* 2010; Borchert *et al.* 2015; Moreau 2016), exerting thus an influence on cambial dormancy (Brienen *et al.* 2016; Nath *et al.* 2016). Both leaf shedding and cambial dormancy are coupled to climatic seasonal variation at varying degrees depending on the leaf shedding habit of the species (Borchert 1999) and the intensity of the climatic variations (Worbes 1995). Yet, the level of phenological adaptation to varying climatic conditions and its impact on wood formation are poorly understood (O'Brien 2008; Couralet *et al.* 2010; Vlam *et al.* 2014).

Trees respond to changing environmental conditions by adjusting their cambial activity, which depends on the species and/or the age of a tree (Wimmer 2002; Fonti *et al.* 2010; De Ridder *et al.* 2013; De Micco *et al.* 2016) and affects the structure of wood anatomical features (Chave *et al.* 2009). A considerable amount of information on tree growth conditions can thus be obtained through the analyses of growth rings and other patterns in the wood tissue (Wiemann 1998; Wimmer 2002; Groenendijk 2014).

Wood anatomy variation is an important source of information on tree adaptation (Gartner 1995; Larson 2012; Beeckman 2016; Tarelkin *et al.* 2016). Studying intraspecific anatomical and phenological variation across climatic gradients or between contrasting sites therefore constitutes a valuable approach in tree growth research (Carlquist 1988; Villar-Salvador *et al.* 1997; Wheeler *et al.* 2007; Fonti *et al.* 2010).

Previous studies have established a close relationship between anatomical features and wood density (Martinez-Cabrera *et al.* 2009; Zieminska *et al.* 2013). Fibres with thicker walls and flattened lumina increase wood density while vessels and parenchyma are negatively correlated to wood density (see previous chapter). Density variations across a pith-to-bark profile are thus a good indicator of the variations of the fibres anatomy and their abruptness. Since the variations of anatomical features are themselves a consequence of a varying cambial activity, pith-to-bark density profiles can be linked to tree growth patterns.

In this study we investigated the effect of precipitation seasonality on leaf phenology and wood formation. We hypothesized that longer and more intense dry seasons lead to increased length of leaf shedding periods and to higher variability in wood anatomical features from pith to bark. Based on previous findings (Borchert 1999), we also assumed a difference in response between deciduous and evergreen species. To test these hypotheses, we used wood density fluctuations as proxies for wood anatomy variability and tree growth patterns of 13 tropical tree species and compared their variability between three sites of the Congo Basin with contrasted precipitation regimes. Pith-to-bark wood density profiles were analysed with wavelet transform (Morlet 1983) and three features summarizing wood formation dynamics were derived. To study leaf phenology, we compiled data from historical databases and compared the synchronicity of phenological events and the lengths of leafless periods of the studied species between two of the three sites. Finally, to study how leaf phenology and wood formation are coupled, we compared the inter-site differences of wood anatomy between different leaf phenologies.

Materials and Methods

Study sites

Samples were collected in the Democratic Republic of the Congo (DRC). Three sites, the Yoko Reserve (0.33°N, 25.31°E), the Bolobo forests of Malebo (2.49°S, 16.50°E) and the Man and Biosphere Reserve of Luki (5.39°S 13.4°E), were selected for their contrasting rainfall regimes to compare the differences in wood formation dynamics (Figure 3-1).

The Yoko reserve has the highest annual precipitation of ~1780 mm (Cassart *et al.* 2016) and the least pronounced dry season (Figure 3-1b). The Biosphere Reserve of Luki and the forests of Malebo have an average annual precipitation of 1100 mm (Figure 3-1b; De Ridder *et al.* 2013) and 1600 mm (Vancutsem *et al.* 2006) with an average of three and four dry months respectively. Relative humidity remains constant throughout the year in Luki, buffering the intensity of the dry season (De Ridder *et al.* 2013). The three soil types are nutrient poor with Xanthic Ferralsol in Yoko (WRB 2014), Ferralic

Arenosol in Malebo and Orthic Ferralsol in Luki (FAO 2007). The plant extractable water capacity is the highest in Yoko (8.77 – 11.65 cm), followed by Luki (6.08 – 7.11 cm) and Malebo with 4.31 – 6.08 cm (Dunne & Willmott 2000).

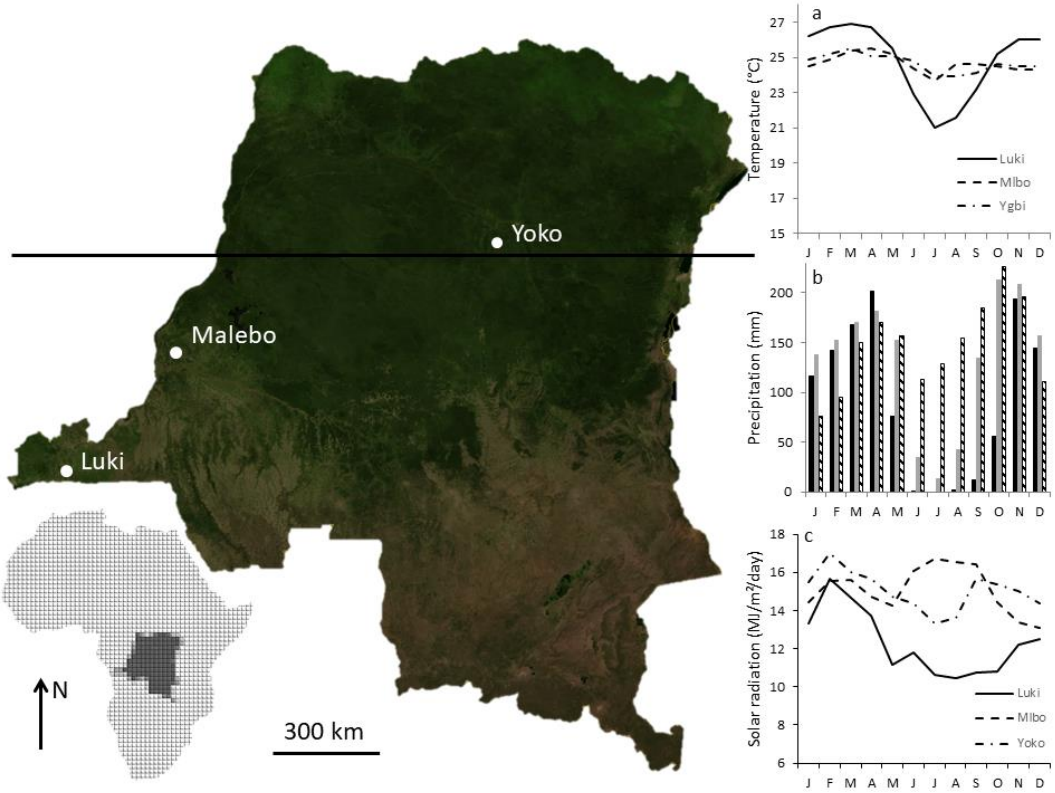


Figure 3-1 : Map of the Democratic Republic of Congo showing the geographical location of the three study sites, a) their monthly average temperature, b) their monthly precipitation and c) the average monthly solar radiation. Source: www.worldclim.org (Hijmans et al., 2005) [accessed 26 June 2017].

Species selection and samples collection

We selected 13 species (Table 3-1) with abrupt anatomical variations on the pith-to-bark axis and with different leaf phenologies. These species are common among the three sites, and cover between 10-20% of the basal area of the forest (Table 3-1). Seven to thirteen trees per species were sampled at breast height with an increment corer (Ø = 5.15mm, Table 3-1). Three to four cores were collected per tree, stored in paper straws and then oven dried for 24h at 103°C. The cores are now registered and stored in the xylarium of the Royal Museum for Central Africa, Belgium (accession numbers: Tw68460 - Tw68881).

Table 3-1 : Selected species with their leaf shedding behaviour, per-site abundance (% of the total basal area), number of sampled trees per site, mean diameter and range (in centimetres), number of trees with phenology records per site and the duration of the observations (in years).

Species	Total basal area (%)			Sampled trees (#)			Diameter range (cm)	Trees with phenology records (#)		Observations span (year)	
	Luki	Mlbo	Yoko	Luki	Mlbo	Yoko		Luki	Ygbi	Luki	Ygbi
[(e)]*Anonidium manii* (Oliv.) Engl. & Diels.	0.01	0.3	2.8	0	10	10	15-30-46	0	2	-	46-56
[(d)]*Canarium schweinfurthii* Engl.	0.4	0.1	0.3	13	10	10	15-42-65	16	5	47-57	38-52
[(e)]*Cola griseiflora* De Wild.	3.4	1	1.1	10	8	10	11-20-30	0	5	-	38-53
[(d)]*Entandrophragma angolense* (Welw.) C.DC	0.3	1.7	0.2	10	8	9	19-34-65	6	4	47-57	38-52
[(e)]*Gilbertiodendron dewevrei* (De Wild.) J. Léonard	0.01	3.3	10.9	10	10	9	17-36-77	0	5	-	38-56
[(e)]*Macaranga spinosa* (Müll Arg.)	0.03	NA	0.5	9	9	11	15-25-40	1	3	47-57	38-53
[(d)]*Milicia excelsa* (Welw.) C.C.Berg	0.1	0.9	0.03	9	9	12	32-51-82	31	6	47-57	37-56
[(e)]*Ongokea gore* (Hua) Pierre	0.1	1.6	0.3	10	8	2	24-41-60	17	3	47-57	38-52
[(e)]*Polyalthia suaveolens* Engl. & Diels.	1.2	4	1.5	10	10	10	20-30-50	50	0	47-57	-
[(e)]*Pycnanthus angolensis* (Welw.) Warb.	0.6	2.9	0.3	10	9	10	23-46-77	27	4	47-57	37-55
[(d)]*Ricinodendron heudelotii* (Baill.) Pierre ex Heckel	1.4	0.3	0.5	9	9	9	22-52-95	46	3	47-57	43-56
[(e)]*Staudtia kamerunensis* Warb.	2.1	1.2	1.2	10	10	10	13-37-65	46	2	47-57	46-56
[(e)]*Tetrorchidium didymostemon* (Baill.) Pax & K.Hoffm.	0.01	0.02	0.1	9	7	10	14-20-33	7	3	47-57	38-55
Total	9.7	17.3	19.7	119	117	122	-	247	45	-	-

Mlbo = Malebo and Ygbi = Yangambi.

(e) = evergreen and (d) = deciduous are leaf phenology data according to the CoForTraits database (Benedet et al., 2015).

Abundance data comes from databases used in Kearsley et al. 2013 (33 ha; Yangambi), Bastin et al., 2014 (35 ha; Malebo) and De Ridder, comm pers. (20 ha; Luki).

Wood density and anatomy analysis

Wood density profiles

The samples were mounted in custom-made cardboard holders and scanned with a resolution of 110 µm using the Nanowood X-ray CT scanner (Dierick *et al.* 2014), developed at the Ghent University Centre for X-ray Tomography (UGCT; http://www.ugct.ugent.be). Top and bottom of the holders were scanned separately, reconstructed with the Octopus software (Dierick *et al.* 2004; Vlassenbroeck *et al.* 2007; licensed by InsideMatters: www.insidematters.eu) and digitally stitched to obtain a single greyscale volume for each holder. Each single wood core was then extracted from the total greyscale volume and its pith-to-bark density was calculated using air density (1.2 kg m^{-3}) and a reference material with known density (1400 kg m^{-3}) and similar elemental composition to wood (De Ridder *et al.* 2011; Bastin *et al.* 2015a; De Mil *et al.* 2016). Structure direction was then manually corrected for ring curvature and radial variations in grain direction (Van den Bulcke *et al.* 2014), based on which a re-interpolated density profile was calculated (De Mil *et al.* 2016).

Wood density profiles as proxies for wood anatomy variation

We used wood density variations as a proxy for the variability of anatomical features within and between individuals from different study sites. Wood density profiles were detrended to avoid tree size and age dependent variations (Briffa & Melvin 2011; Peters *et al.* 2015) by subtracting a smoothed profile obtained with a moving average of 50 density values. The detrended profiles were analysed with wavelet transform (Morlet 1983) and three descriptors summarizing different aspects of wood formation were derived. These descriptors were then compared between individuals, between species and between sites.

Wavelet analysis allows processing complex signals with varying amplitudes and frequencies (Lau & Weng 1995). It is a powerful tool for the identification of the main periodicities within a signal and the evolution of amplitude, frequency and phase (Farge 1992; Rigozo *et al.* 2005). The wavelet analysis overcomes the main limitation of the Fourier transform that only identifies the frequencies present in

a signal but not their localisation in time (Schaefli & Zehe 2009). It decomposes the signal into portions of variable length and convolutes them with wavelets generated from an original (mother) wavelet (Farge 1992). The covariance between the two is calculated and the obtained values allow generating a space-frequency representation of the signal (Figure 3-2; Schaefli & Zehe 2009).

With D(x) the density profile and ψ the mother wavelet, the continuous wavelet transform (CWT) is expressed as:

$$CWT(u,s) = \frac{1}{\sqrt{s}} \int D(x)\, \psi\left(\frac{x-u}{s}\right) dx$$

Where *u* and *s* represent respectively the space translation and the scaling factor of the mother wavelet. The scale factor can be expressed in physical units (centimetres) and measure the width of one density fluctuation.

A Ricker wavelet, also known as Mexican hat (Ricker 1953), was used as the mother wavelet. This wavelet shape has the advantage to enhance local peaks of wood density surrounded by lower density area, with a predefined scale. Several scale factors, each representing the sizes of the wavelets, were tested.

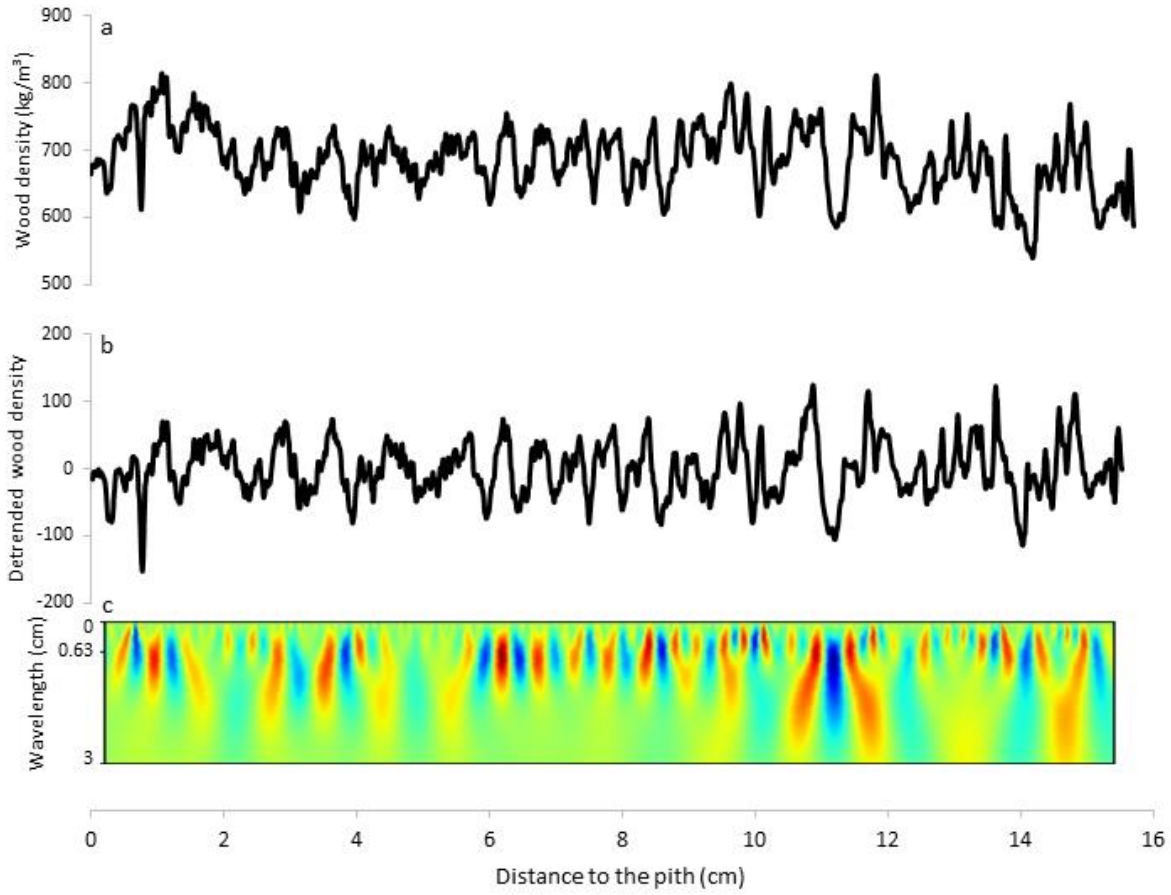


Figure 3-2 : Superposition of a wood density profile and the correspondent space-frequency representation. In a) wood density is measured with 3D X-ray tomography with a 110 µm resolution to obtain a pith-to-bark profile. This profile is then detrended in b) and the result is analysed with a Ricker wavelet of wavelengths varying from 0 to 3 cm to generate a space-frequency representation of the density profile in c). Different shades of red/blue indicate a positive/negative correlation between the wavelet and the signal portion while the green background represents no correlation. The intensity of the coloration depends on the amplitude of the variation of the density signal.

A space-frequency representation of the signal (Figure 3-2c) is only qualitative, therefore, three features of the signal, each quantifying a wood density variations pattern, were calculated:

a) The dominant wavelength of wood density fluctuation

Wood density profiles consist of density fluctuations of varying width that are more or less correlated with wavelets generated from a mother wavelet by dilation or compression (Farge 1992). Wavelengths of certain width contribute more to the power spectrum throughout the profile, which enables us calculating the dominant wavelength of density fluctuations in the studied tree. This dominant

wavelength reveals the average width of a growth zone in a tree and is represented by the position of the peak in Figure 3-3a.

The dominant wavelength was expressed as the scale position of the maximum CWT coefficients values, regardless of the spatial axis:

$$\{x|\ \forall y : \sum_{u} CWT^2(u,y) \leq \sum_{u} CWT^2(u,x)\}$$

b) Frequency distribution representing the regularity of wood formation

Related to the first descriptor, the second one estimates the regularity with which wood density fluctuates from pith to bark. Trees forming growth zones of same width need a lower number of different wavelets to characterize their density profile compared to trees with growth zones of varying widths from pith-to-bark. This second descriptor was estimated by calculating the cumulated power of the wavelets with wavelengths from 0 to a threshold value *th* (the area under the curve in Figure 3-3b):

$$\sum_{s=0}^{th}\sum_{u} CWT^2(u,s)$$

The threshold value *th* was arbitrary set to wavelength of 2cm as this captured most of the variability of wavelengths in the density profiles (Figure 3-2c).

c) The amplitude of wood density variation

Besides the rhythmicity of wood density variation, the amplitude is a useful descriptor for comparing the distinctness of growth zones between trees. It can be estimated with the power of the spectrum of the dominant wavelength (Figure 3-3c) and can be calculated using the square of weight of the energy of the density signal at the main frequency of the wavelets:

$$\frac{1}{N}\Sigma_{i=0}^{N}\left(d * R(f)\right)^2$$

Where the '*' operation is a convolution, d is the wood density profile with N measures of density and R is the Ricker function at frequency f.

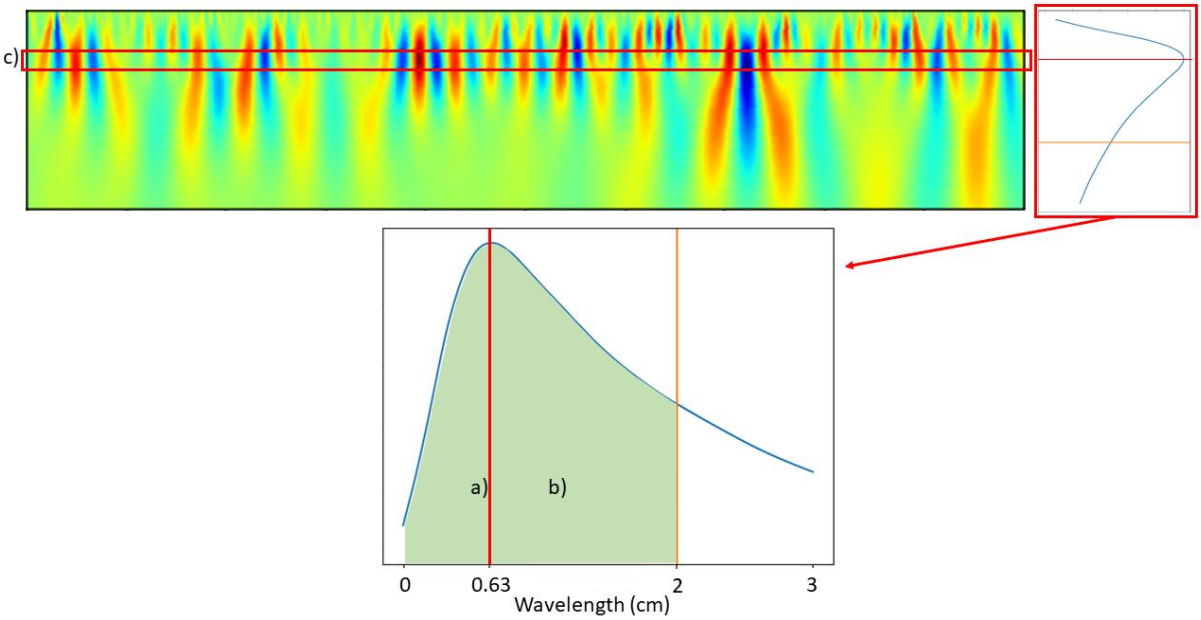


Figure 3-3 : Visual representation of the wood density profiles descriptors derived from the wavelet analysis: a) the dominant wavelength determining the main periodicity of wood density fluctuation (illustrated by the position of the peak), b) the frequency distribution determining the regularity of wood formation (illustrated by the area under the curve) and c) the amplitude of wood density fluctuations (illustrated by the intensity of the red/blue coloration).

Leaf phenology

We used historical data collected by the former Institut National pour l'Etude Agronomique du Congo belge (INEAC) at both Luki and Yangambi (±100 km to the North-West of Yoko with similar precipitation patterns) to compare leaf phenology between two contrasting climates and to the CoForTraits database (Benedet *et al.* 2015). Various numbers of trees of each species were observed every ten days in the period 1947 – 1957 in Luki (Couralet *et al.* 2013) and every week in the period 1939 – 1958 in Yangambi (Table 3-1, www.cobecore.org). The mean probability of observing a leafless tree for the n^{th} week (or ten days period) of the year was calculated by averaging each year's observations for the n^{th} week. We then calculated the average calendar date of leaf fall, flushing and the temporal aggregation of both events for each species in both sites using circular statistics (Batschelet 1981;

Milton 1991; Davies & Ashton 1999). Dates were considered as angles $Ø_i$ between 0° and 360° and the mean angle $\overline{Ø}$ was calculated as:

$$\overline{Ø} = arctan\left(\frac{y}{x}\right) \text{ if } x > 0 \quad \text{or} \quad \overline{Ø} = 180 + arctan\left(\frac{y}{x}\right) \text{ if } x < 0$$

where

$$x = \frac{1}{n}\sum cosØ_i \text{ and } y = \frac{1}{n}\sum sinØ_i$$

with the number of trees ranging from 1 to n. The temporal concentration or synchronicity (r) allows estimating the degree of coordination between trees for leaf shedding or flushing:

$$r = \sqrt{(x^2 + y^2)}$$

Finally, we divided the number of years with observed leaf fall by the total number of years to calculate the probability for a tree of a given species to shed leaves during a year.

Leaf phenology and wood density variability analysis

The number of wood cores per tree varying between two and four, wood density profiles descriptors were averaged at the tree level. The obtained values were then compared between species, sites and leaf phenologies with Kruskal-Wallis and post-hoc Dunn tests. The interaction between site and leaf shedding behaviour was also tested. All analyses were carried out in R 3.3.1 (R Core Team 2016).

Results

Wood anatomy, wood density and wavelet analysis

The relationship between wood anatomy and wood density (Figure 3-4, Supporting information Figure S1)) shows that wood density is mostly driven by the fibres anatomy. Zones of flattened fibres with thickened cell walls are translated into density peak on the pith-to-bark profiles. Growth rings defined by flattened fibres and/or thickened walls can clearly be located on a density profile (Figure 3-4a, d, f). The effects of parenchyma and vessels on wood density are less pronounced and it is difficult to locate

a variation of these tissues with the help of a density profile. Profiles with the most abrupt density fluctuations show the lowest peak position values. An irregular rhythmicity in density fluctuation results in wider frequencies distribution (and thus greater values). Finally, density profiles with the most frequent and pronounced density fluctuations have the highest values of power spectrum. On average, the intra-individual variability of wood density descriptors is of 15% with some individuals showing around 50 % of variability within.

Although conspecific trees from different sites are anatomically alike, some details do vary among sites (*e.g.* Figure 3-4a, b). In general, trees growing in Luki and Malebo exhibit more pronounced variations in fibre diameter or wall thickness along the pith-to-bark profile. The vast majority of differences occur mainly at the level of fibres with only one species showing differences in vessel density variation (*Ongokea gore*) or parenchyma density variation (*Cola griseiflora*). Illustrations for all species from each site are given in the Annex 3.

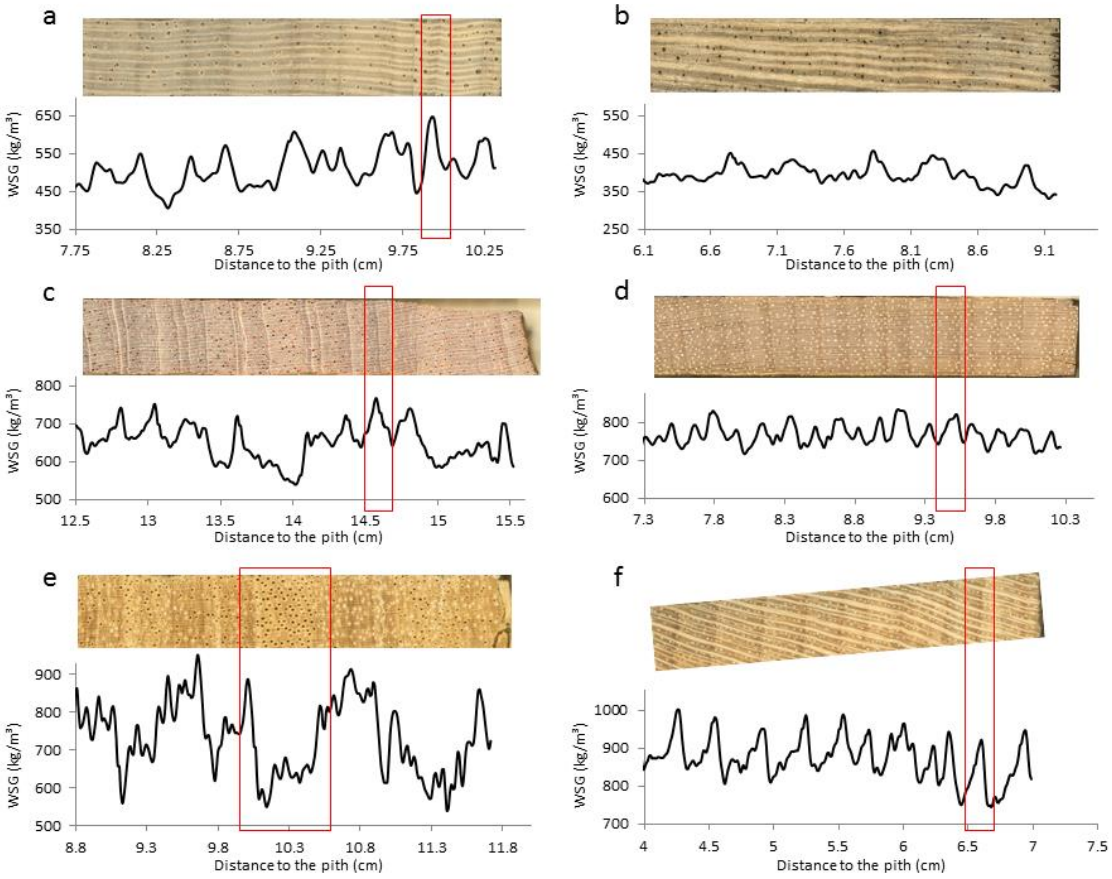
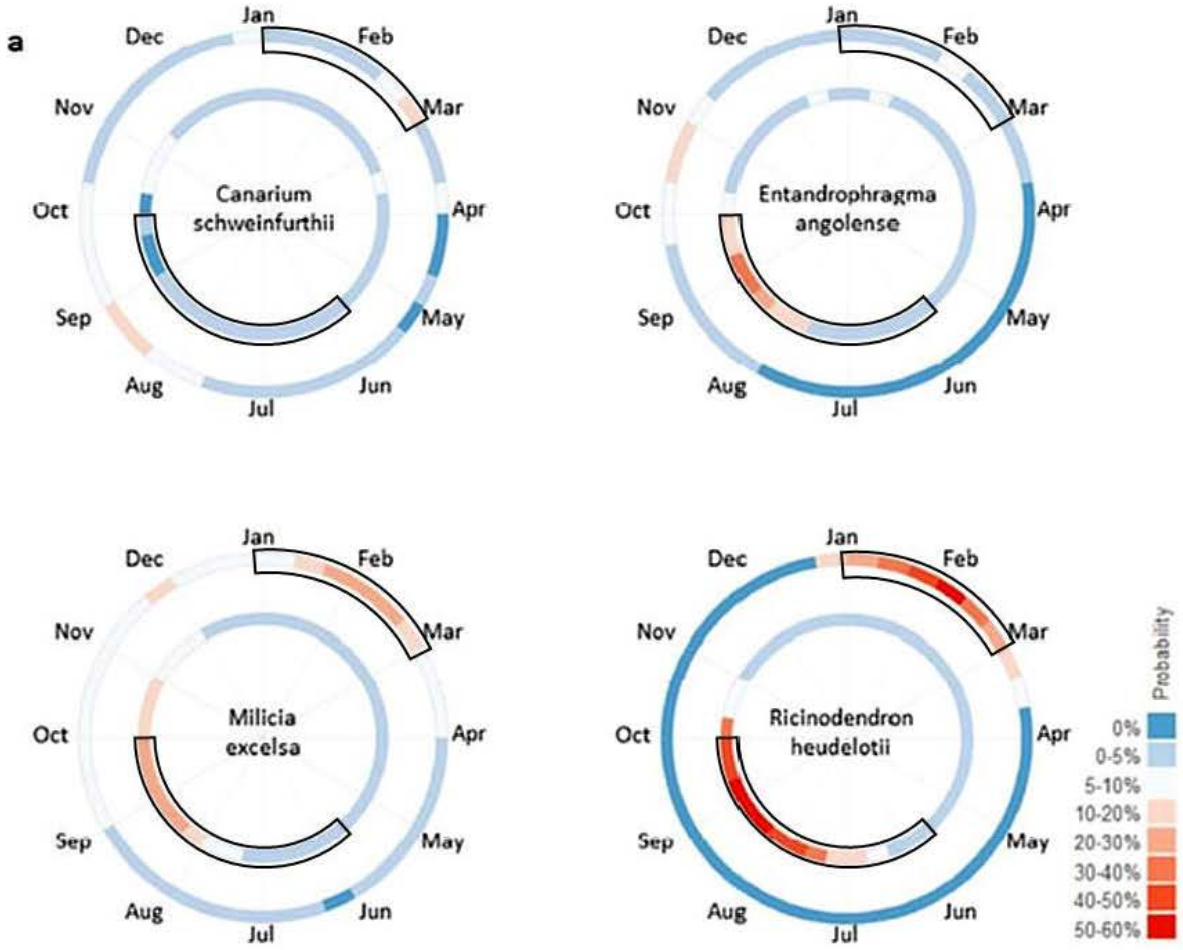


Figure 3-4 : Illustration of the relationship between wood density and wood anatomy. Representation of the last 3 cm under bark of transversal surfaces of a) A. mannii from Malebo, b) A. mannii from Yoko, c) E. angolense from Malebo, d) S. kamerunensis from Luki, e) O. gore from Malebo and f) P. suaveolens from Luki. Density peaks correspond to darker zones defined by flattened fibres and/or thickened fibre walls (red rectangles in a, c, d and f). The red rectangle in e points to a density decrease driven by an increase in vessel density. In a, c, d and f, the observed patterns are similar to the most common configurations of growth ring boundaries (see Tarelkin et al 2016).

Leaf phenology

Phenology records for all species (Figure 3-5) are concordant with the CoForTraits database (Table 3-2). In Luki, trees show more synchronicity and longer periods of leaf shedding than in Yangambi (Table 3-2). However, the probability to observe a leafless tree of a deciduous species during the dry season is similar in Luki and Yangambi (Figure 3-5a). The evergreen species, with the exception of *Tetrorchidium didymostemon*, show weak differences between sites and the probability of leaflessness is low in both sites (Figure 3-5b). Although some trees of evergreen species show long leafless periods in Luki (*Pycnanthus angolensis* – 115 days, *Ongokea gore* – 50 days), the number of years with leafless events is very low (<20%) compared to the observations of deciduous species (>80%, Table 3-2).


a
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Canarium schweinfurthii
Entandrophragma angolense
Milicia excelsa
Ricinodendron heudelotii
Probability
0%
0-5%
5-10%
10-20%
20-30%
30-40%
40-50%
50-60%

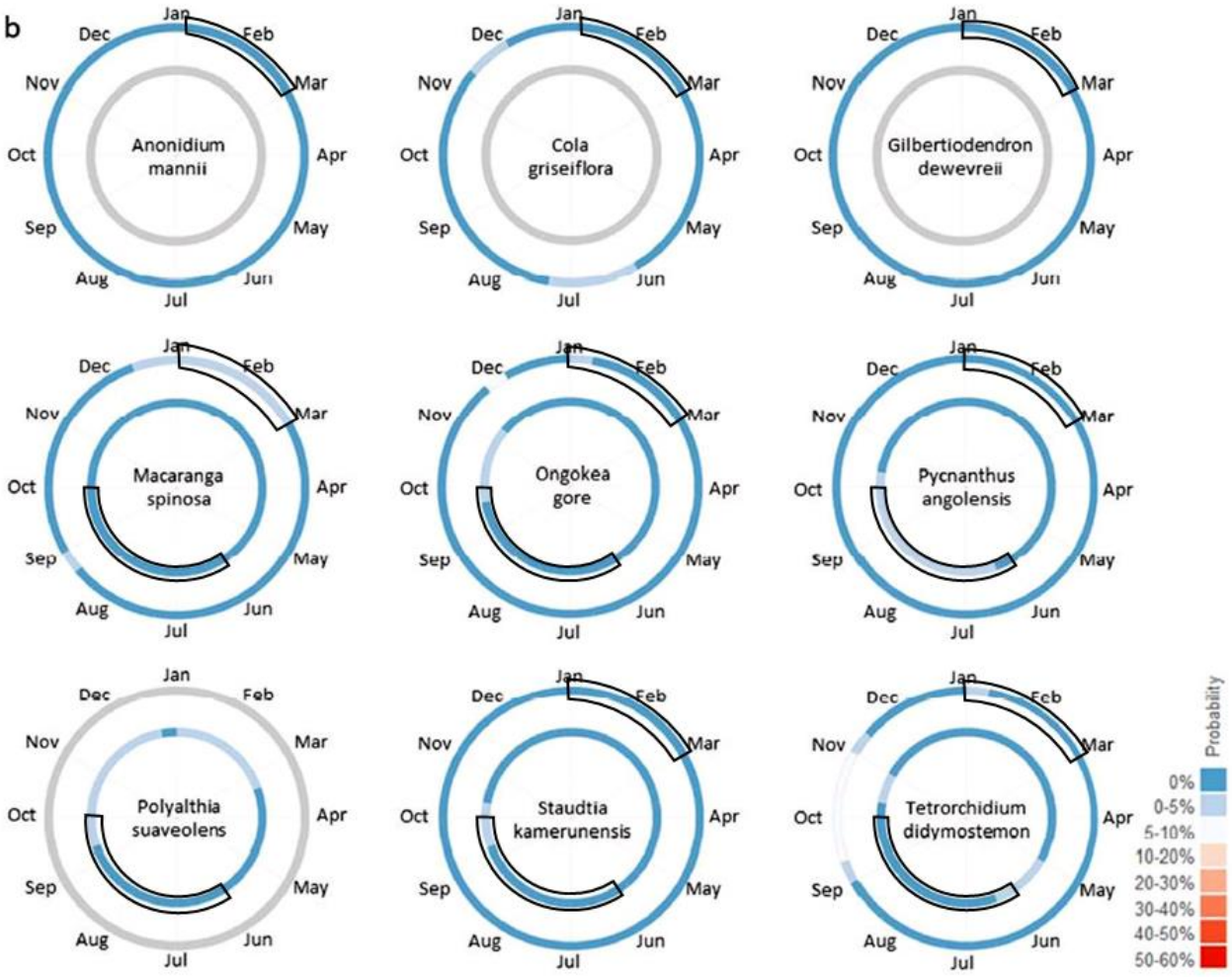


Figure 3-5 : Leaf shedding behavior of a) deciduous and b) evergreen species in Luki (inner circle) and Yangambi (outer circle). The red-blue coloration gradient represents the probability to observe a leafless tree of the studied species during the corresponding period of the year. The respective dry seasons of both sites are highlighted in black: June-September for Luki and January-February for Yangambi. Missing observations are represented in grey.

Table 3-2 : Summary of the leaf shedding behaviour in Luki and Yangambi: mean length of the leafless period, inter-trees synchronicity of leaf fall and flushing and the proportion of years with observed leaf shedding averaged at the species level.

Species	Length of leafless period (days)		Synchronicity (r)		Proportion of leafless years	
	Luki	Yangambi	Luki	Yangambi	Luki	Yangambi
[(e)]*Anonidium mannii*	NA	0	NA	/	NA	0 (2)
[(e)]*Cola griseiflora*	NA	22.56	NA	0.44	NA	0.06 (5)
[(d)]*Canarium schweinfurthii*	13.30	7.98	0.31	0.15	0.31 (16)	0.65 (5)
[(d)]*Entandrophragma angolense*	34.79	13.13	0.71	0.55	0.55 (6)	0.52 (4)
[(e)]*Gilbertiodendron dewevreii*	NA	0	NA	/	NA	0 (5)
[(d)]*Milicia excelsa*	46.93	33.91	0.62	0.39	0.59 (31)	0.83 (6)
[(e)]*Macaranga spinosa*	0	10.05	/	0.59	0 (1)	0.11 (3)
[(e)]*Ongokea gore*	50	7.5	1	0.95	0.01 (17)	0.06 (3)
[(e)]*Pycnanthus angolensis*	115	0	1	/	0.01 (27)	0 (4)
[(e)]*Polyalthia suaveolens*	29.26	NA	0.83	NA	0.02 (50)	NA
[(d)]*Ricinodendron heudelotii*	79.93	32.13	0.81	0.95	0.64 (46)	0.84 (3)
[(e)]*Staudtia kamerunensis*	30	0	1	/	0 (46)	0 (2)
[(e)]*Tetrorchidium didymostemon*	40	33.75	0.26	0.71	0.03 (7)	0.07 (3)

(e) = evergreen and (d) = deciduous species according to the CoForTraits database (Benedet et al., 2015).

NA = data non available

/ = no leaf shedding during the observation period

The numbers between the brackets in the last two columns are the total number of trees in the database of the observation

Leaf phenology and density variability

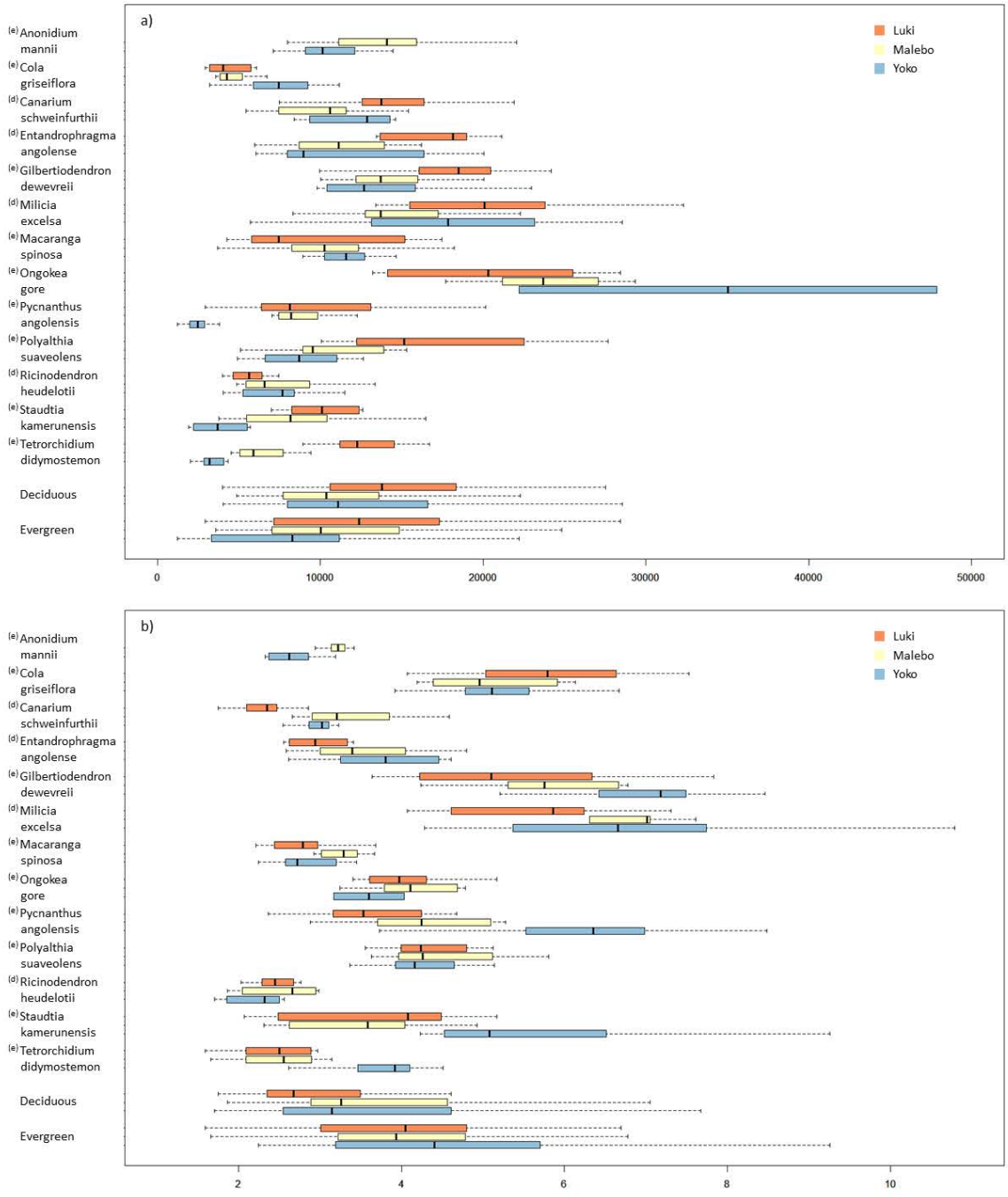
Results of the Kruskal-Wallis/Dunn's comparison tests show that leaf phenology influences the main periodicity of wood density fluctuation (dominant wavelength), while both the site and leaf phenology influence its regularity (frequency distribution) and the amplitude of anatomical variation (Table 3-3). Deciduous species exhibit density fluctuations with higher amplitudes and narrower widths. Dunn's pairwise comparisons of density profiles descriptors between sites (Table 3-3) and Figure 3-6a reveal that the amplitude of density fluctuation is higher in Luki than in less seasonally dry sites such as Malebo and Yoko. Additionally, wood density rhythmicity is significantly more regular in Luki and Malebo than in Yoko (Figure 3-6b). No significant influence of precipitation seasonality was found for growth periodicity (Figure 3-6c). Drier conditions and more pronounced deciduousness appear thus to increase the regularity of the cambial activity and the abruptness of the anatomical variations.

Site and phenology effects also interact to influence the amplitude of density fluctuation, which is significantly lower in evergreen species from Yoko than in the two other sites, while deciduous species show no difference between sites. The wood formation of evergreen species seems more sensitive to differences in precipitation patterns than the deciduous ones.

Table 3-3 : Summary of the comparison of the density profile descriptors between the studied sites and leaf phenology. P-values are calculated with the Kruskal-Wallis test.

Grouping factor	Growth descriptor	Mean (SD)	p-value
Site	Peak position	L: 0.67 (0.13) M: 0.66 (0.1) Y: 0.67 (0.08)	0.3
	Peak width	L: 3.79 (1.44) M: 4.01 (1.34) Y: 4.47 (1.9)	<0.05
	Power spectrum	L: 13791 (7326) M: 11446 (6056) Y: 9948 (6824)	<0.001
Phenology	Peak position	D: 0.69 (0.08) E: 0.65 (0.11)	<0.001
	Peak width	D: 3.75 (1.82) E: 4.27 (1.47)	<0.001
	Power spectrum	D: 12814 (6075) E: 11158 (7255)	<0.01

L = Luki, M = Malebo and Y = Yoko; D = Deciduous and E = Evergreen


a)
Luki
Malebo
Yoko
(e) Anonidium mannii
(e) Cola griseiflora
(d) Canarium schweinfurthii
(d) Entandrophragma angolense
(e) Gilbertiodendron dewevreii
(d) Milicia excelsa
(e) Macaranga spinosa
(e) Ongokea gore
(e) Pycnanthus angolensis
(e) Polyalthia suaveolens
(d) Ricinodendron heudelotii
(e) Staudtia kamerunensis
(e) Tetrorchidium didymostemon
Deciduous
Evergreen
0
10000
20000
30000
40000
50000
b)
Luki
Malebo
Yoko
(e) Anonidium mannii
(e) Cola griseiflora
(d) Canarium schweinfurthii
(d) Entandrophragma angolense
(e) Gilbertiodendron dewevreii
(d) Milicia excelsa
(e) Macaranga spinosa
(e) Ongokea gore
(e) Pycnanthus angolensis
(e) Polyalthia suaveolens
(d) Ricinodendron heudelotii
(e) Staudtia kamerunensis
(e) Tetrorchidium didymostemon
Deciduous
Evergreen
2
4
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8
10

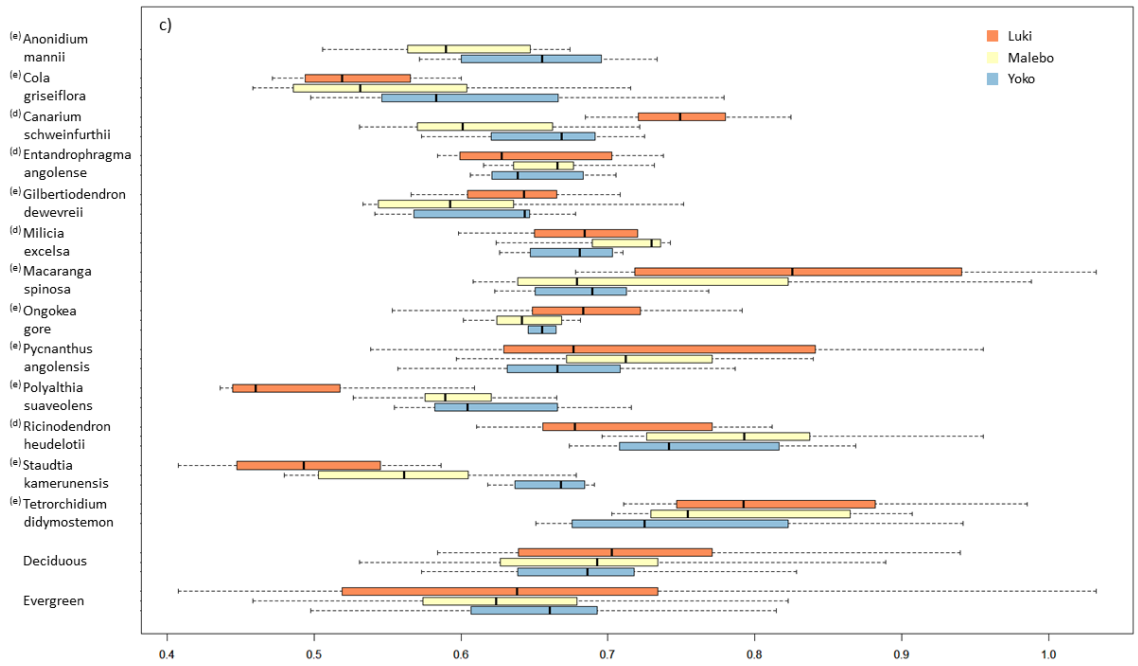

Figure 3-6 : Intersite differences of the values of a) amplitude of density fluctuations, b) frequency distribution and c) the dominant wavelength of the pith-to-bark wood density variations. Species leaf phenology is indicated by (e) = evergreen species and (d) = deciduous species.

Discussion

Our study takes place in the framework of the research on tree growth in varying environmental conditions. We explored intra- and inter-species differences in leaf phenology and wood anatomy along a precipitation gradient crossing the critical precipitation threshold of less than 50mm per month during dry months (Worbes 1995; Worbes 2010; Fichtler & Worbes 2012). Previous studies combined wood density and wood anatomy data and established a strong link between both (Zanne *et al.* 2010; Zieminska *et al.* 2013). In our study we show for the first time, for a large selection of African tropical tree species, that it is possible to quantify several aspects of pith-to-bark wood density profiles and, therefore, synthesize various aspects of tree growth (*i.e.* cambial activity rhythmicity, abruptness of anatomical variations). More so, the metrics derived from the wavelet analysis allow to easily compare wood anatomical and growth dynamics within and between plant functional types (*i.e.* species, phenologies, etc…). Yet, due to the integrative nature of the wood density it remains difficult to disentangle the relative contribution of different anatomical features for the observed variability of wood density.

Wood anatomy and growth regularity

The deciduous species showed a higher variability in their anatomical features and more consistent intra-annual growth patterns as indicated by the higher amplitudes and the more constant frequencies of density fluctuations. Various studies have explored the link between deciduousness, cambial activity (O'Brien *et al.* 2008; Brienen *et al.* 2016) and growth-ring distinctness (Lisi *et al.* 2008; Nath *et al.* 2016). Deciduous trees are more likely to form distinct growth rings with abruptly varying anatomical features in response to stress although several evergreen species forming distinct growth rings have been reported too (Carlquist 1988; Callado *et al.* 2001; Marcati *et al.* 2006) and are present in our study (e.g. *S.kamerunensis*, *P. suaveolens* which are among the most common species of Central Africa; Bastin *et al.* 2015b). Moreover, O'Brien *et al.* (2008) found a negative correlation between leaflessness and diameter growth patterns. Trees shedding leaves once a year are thus likely to show more regular patterns of wood density fluctuation.

Our results indicated a higher inter-site variability of anatomical features from pith to bark for evergreen species than for leaf-shedding species, with higher variability in the drier sites (Luki, Malebo) compared to the wettest (Yoko). It suggests that even though evergreen species don't generally form distinct growth-rings they still modulate their cambial activity according to environmental conditions (Borchert 1999; O'Brien *et al.* 2008) and that wood anatomy reveals more subtle responses to climate than leaf phenology. The longest dry seasons in Luki and Malebo, coupled to the lowest plant extractable water capacities of the soils cause a periodical water stress that impacts diameter growth (Alvim 1964; Borchert 1999; Lisi *et al.* 2008) and increases variability in anatomical features. Our results hereby corroborate previous research along a precipitation gradient in Costa Rica which showed that *Gmelina arborea* trees growing in sites with less precipitation had the highest density variability due to more distinct growth rings (Moya & Filho 2009).

Moreover, trees from drier sites also showed more regular growth patterns (the frequency of wood density fluctuations remains constant throughout the entire core). The lack of strong seasonal growth

limiting factors not only reduces the variability in wood anatomy but also affects the interannual regularity of tree growth. Leaf shedding is also less regular and synchronized in Yangambi, indicating that tree growth patterns vary from year to year and between individuals. Deciduousness is associated with several tree functions, such as carbon sequestration, reproductive events (Singh & Kushwaha 2016) and its lack of synchronicity could be linked to the interannual irregularity of growth (O'Brien *et al.* 2008).

Leaf phenology

Leaf phenology differs significantly between Luki and Yangambi. The peak of leaf shedding for deciduous species occurs by the end of the respective dry seasons in both sites. These results corroborate previous studies that link leaf shedding to seasonal droughts (Borchert 1999; Couralet *et al.* 2013; Brienen *et al.* 2016; Nath *et al.* 2016). The leaf phenology patterns detected in Luki and Yangambi differed by the intensity and the length of leaf shedding. All deciduous species shed leaves for a longer period in Luki and were more synchronous in shedding. These differences may be explained by the longer and more intense dry season in Luki leading to an increased leaf shedding to reduce evapotranspiration (Borchert 1999). Moreover, several authors reported an insolation-decrease-driven leaf shedding in tropical forests (Biudes *et al.* 2015; Borchert *et al.* 2015; Moreau 2016). Luki is the southernmost site and the dry season of June-September to the shortest photoperiod. The decrease in day length and a thick cloud cover during the dry season in Luki negatively affects the light quantity (duration x intensity) and might intensify leaf shedding. A similar trend for evergreen species was noticed but only concerned very small numbers of trees that are probably not representative of the leaf shedding behaviour of the entire population. Moreover, there is a continuous spectrum of varying leaf-shedding patterns between deciduous and evergreen species in the Tropics (Singh & Kushwaha 2016) and some evergreen species experience deciduousness in certain conditions.

A strong intra-species intra-site variation in leaf phenology was observed. Couralet *et al.* (2013) reported that, in average, the proportion of leafless trees rarely exceeded 50% per species in Luki, even for the deciduous ones. Moreover, large differences in the synchronisation of leaf phenology events, consistent throughout the seasons, were also reported in Luki (Couralet *et al.* 2013; De Mil *et al.* unpublished) and other regions (Borchert *et al.* 2015). The lack of synchronicity in leaf shedding and flushing complicates any generalization about environmental drivers of leaf phenology (Viennois *et al.* 2013) and indicates that site dependent micro-environmental variations strongly influence leaf phenology and tree growth (De Frenne & Verheyen 2016; Singh & Kushwaha 2016). Due to the historical nature of the phenology data used in this study, it is difficult to characterize site-dependent micro-environmental conditions of tree growth. Yet, the large temporal span and the number of trees described in these databases represent a rare and valuable source of information within forest ecology and a unique opportunity to assess the variation of leaf-shedding in the last 70 years.

The predicted shifts of precipitation regimes due to climate change (several models agree on increased drought in the Congo Basin; James *et al.* 2013) will influence tree growth and it is critical to assess its impact on tropical forests. Our results suggest a higher sensitivity to differences in growth conditions of evergreen species. They showed the highest anatomy variability in sites with the most pronounced seasonality indicating that evergreen tree growth might be more affected by changing environmental conditions. However, these results are to be interpreted carefully as studying the influence of temporally varying environmental conditions by analysing the geographically-driven differences only gives an approximation of the studied environmental variations. While such an approach reveals how conspecific individuals have adapted to different environmental conditions, it leaves no information on how they would adapt to fast climatic changes (James *et al.*, 2013). Moreover, other environmental variables are expected to vary in the climate change framework, such as temperature, the intensity of climatic events or the CO_2 concentration (IPCC, 2014). Their influence on tree growth are subject to debate and need to be researched more thoroughly (Zuidema *et al.*, 2013; Van Der Sleen *et al.*, 2015; Brienen *et al.*, 2016). Further exploration of tree species plasticity in cambial regulation and ultimately

carbon allocation should provide more information on the effects of climate change on tropical forests. Ongoing research by De Mil and colleagues (unpublished) showed encouraging results in estimating past cambial activity and leaf phenology based on intra-annual wood anatomy. Periodic cambial wounding proved its efficiency in tree growth studies (Mariaux & Bossanyi 2016) and could help linking different climatic events to wood anatomy. The integration of these techniques with a more thorough description of micro-environmental growth conditions and long-term climate records should provide useful insights in tree growth – climate change relationship.

Reflections on methodology

Fonti *et al.* (2010) stressed the importance of studying wood anatomical variability in tree growth research. However, the authors warned about the risks of analysing too few anatomical features which might lead to obscured or reduced ecological information. Fast CT scanning of wood cores and wavelet analysis are powerful tools for analysing density profiles and wood anatomical variation of a large number of samples. Automation also reduces subjectivity of visual assessments of anatomical features while limiting the required labour cost. Our methodology, therefore sidesteps in part the issue of historically labour-intensive methodologies that hamper the efforts of the scientific community of acquiring ecologically significant number of samples (Brodribb 2017).

Furthermore, over the last 30 years, wavelet analysis has proven very effective in handling complex, noisy and non-stationary data (Bullmore *et al.* 2003; Hudson *et al.* 2010). It has successfully been used in studies of temperature and rainfall shifts (Palus *et al.* 2005; Koch & Markovic 2007), changes in vegetation cover (Lu *et al.* 2007), plant flowering (Hudson *et al.*, 2010) and tree-ring series (Rigozo *et al.* 2005). However, to the best of our knowledge, wavelet analysis has not yet been applied in wood density studies, although these datasets present characteristics that make them good candidates for this type of analyses. Our study showed that wavelet analysis has the potential to reveal wood formation dynamics and resource allocation changes over the years and to provide effective tools for studying the climate impact on tree growth.

While it is tempting to link the fluctuation patterns of xylem features to climate records, our dataset contains limitations currently hampering that approach. Since trees' annual growth rates are not constant, a transposition of a density profile to a time grid necessitates an adapted approach (Verheyden, 2004). The anchor-point method fixes annual reference points on a timeline (Paillard *et al.*, 1996) allowing thus to superimpose the density profiles over the climate data. This method was successfully applied by Verheyden *et al.* (2005) who linked vessel variations profiles to climate data with a Fourier transformation. However, the annual nature of the observed rings in our sample set and the seasonality of their formation are still to be confirmed. Although the observed mean ring widths in our sample set (0.4 – 1 cm) are within the range of annual diameter increments reported in the literature (Lisi *et al.*, 2008; O'Brien *et al.*, 2008), additional studies are needed to confirm that rings are annual and to determine which moment of the year they are formed. Moreover, a higher temporal resolution is needed in order to account for differences in intra-annual growth rates (De Ridder *et al.* 2004) and study the effects of seasonal variations of climate. We advocate in favour of monitoring cambial activity and leaf phenology with sub-annual resolution (with dendrometers, cambial wounding, micro-coring; see De Mil 2017), which will help linking data such as wood density profiles to climate records and studying the influence of climate on tree growth.

Conclusion

In this study we investigated the relationship between precipitation seasonality, leaf phenology and pith-to-bark variability of wood anatomy. We showed that anatomical features vary more abruptly, more regularly and that trees shed their leaves for longer periods under environmental conditions with distinct seasonality. Leaf shedding differed among species and growth habit. While deciduous species shed leaves more in the driest site they didn't show significant differences in wood structure between sites. On the other hand, evergreen species showed a higher variation in wood anatomy across sites, indicating a higher sensitivity to differences in climatic conditions. Although several general trends of inter-site and inter-species growth differences could have been shown, our study also highlighted the large intra-site and intra-species variability, stressing the importance of describing the micro-

environmental conditions in further studies. By proposing new methods for studying tree growth with wood density profiles, our study opens interesting perspectives in the study of the tropical forests and on carbon stocks variations.

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Thesis discussion

We aimed throughout this thesis to explore the radial variability of wood anatomical features and wood density as well as their intra- and inter-species differences. It is essential to obtain reliable data on trees responses to changing environmental conditions to predict the fate of the tropical forests and their capacity to provide services (Phillips *et al.*, 2010; Gebrekirstos *et al.*, 2014). The greatest limitations so far are a too coarse temporal resolution of studies (Zuidema *et al.*, 2013) and the small number of confirmed annual distinct growth rings suitable for dendrochronology (Rozendaal & Zuidema, 2011). Given that trees' responses to their environment are recorded in the xylem throughout their life (Wimmer, 2002), it is important to increase our efforts to decode this archive by studying wood anatomy variations and to link them to the environmental drivers.

This dissertation focuses on three issues currently encountered in tree growth studies: the lack of uniformity in the definition of tree-ring distinctness, the tedious nature of wood anatomical measurements and the difficulty of assessing and comparing the tree growth variability under different environmental conditions. In this section we discuss our main results and their consequences for tropical forest studies. We then discuss the potential application of our approach, especially targeting the use of wood density profiles in tree growth studies. Finally, we conclude by the 'key findings' as a take-home message.

- Study of growth-ring distinctness of tropical trees and the anatomical features that influence their variability

In Chapter 1 we browsed the RMCA collection of tropical woody specimens and analysed the anatomy of growth-ring boundaries of 103 species and assessed two possible drivers of its variability: the site effect and leaf phenology. While the nature of our sample set prevented us from effectively addressing the more ecological questions, we could highlight the variability of tree-ring boundary distinctness within and between individuals and species. Doing so, we showed that insufficient precision in characterizing tropical growth-rings boundaries may lead to high levels of uncertainty for many species. Combined with the high level of subjectivity of growth-ring distinctness description resulting

in large discrepancies between different studies (Fahn, 1962; Cherubini *et al.*, 2013) it is difficult to construct a unified database that grasps all the complexity and variability of the distinctness of tree-ring boundaries.

Furthermore, we reminded that the criteria to define tree-ring distinctness remain subject to debate. We highlighted several parts of the IAWA definition (IAWA Committee, 1989) that are open to interpretation and might have led to debatable descriptions of ring boundaries distinctness of several tropical species. Moreover, this description fails to take in account tree growth conditions, which may heavily influence growth patterns (Worbes, 1995; Groenendijk *et al.*, 2014). Studying growth rings in the Tropics with the same criteria as in the temperate zones might lead to an underestimation of the number of tropical species with growth rings sufficiently distinct for dendrochronological studies. Indeed, we showed that an important number of tropical species' ring boundaries are defined by other features than flattened fibres or ring porosity (*e.g.* parenchyma, rays). Moreover, the diameter of fibres varies less abruptly in the tropical species, making thus the flattened fibres more difficult to detect and reinforcing the impression that ring boundaries in the tropics are less distinct compared to the temperate species (Tarhule & Hughes, 2002).

To overcome the subjectivity issue, we advocate in favour of the elaboration of more quantitative methods. We proposed defining ring-boundary distinctness based on the ability to assign each cell to the n^{th} or the n^{th+1} ring or elaborating different metrics quantifying ring-boundary distinctness (*e.g.* based on the characterisation of the degree of anatomical variation from pith to bark). However, these methodologies are labour intensive and time-consuming and questions concerning the necessity of such a precision in classical dendrochronology studies may arise. Moreover, they don't address other current issues in tropical dendrochronology: the lack of information on the annual character of rings, circumferential variability, intra-annual fluctuations, wedging or missing rings (Worbes, 2002), etc. It appears thus that other approaches need to be developed to supplement classical dendrochronology that relies on annual tree-ring series.

In the last few years, research has been increasingly directed towards complementing traditional tree-ring width studies with intra-annual information (Fonti *et al.*, 2010; von Arx *et al.*, 2014; De Mil *et al.*, 2017). Trees respond to fluctuating environmental conditions which causes the formation of intra-annual fluctuation of xylem features such as vessels or fibres (De Micco *et al.*, 2016). The most commonly described are intra-annual density fluctuations, called IADF's. Although they were often considered as anomalies hindering proper crossdating during dendrochronological studies, they can be useful markers for unusual climatic events (Brauning *et al.*, 2016). Moreover, they can provide additional environmental information to the information obtained from tree-ring width (Novak *et al.*, 2013) and help unravelling the climate – tree growth relationship with a higher temporal resolution.

Our study showed that growth-ring distinctness and anatomy varies not only between species but also between conspecific individuals. The limitations of our samples set hindered a more ecological approach in this study. Wood cores and/or wood disks have a higher potential in dendroclimatological studies. We also questioned the accuracy of current definitions of growth-ring distinctness and introduced the opportunity to sidestep the issue of the lack of growth-ring distinctness of tropical species: studying continuous pith-to-bark variations of wood anatomy and IADF's. To do so, there is a need for methodological improvements in wood anatomy analysis and tree monitoring. We advocate for a further development of the study of intra-annual variability of wood anatomy. A better understanding of the drivers of the anatomical variability and its relationship with wood density would greatly improve our knowledge of tree growth in the tropical environments. This objective was pursued throughout the Chapters 2 and 3 of this dissertation and the obtained results will be discussed in the following paragraphs of this chapter.

- Study of the wood anatomy – wood density relationship and the use of wood density as a proxy for wood anatomy

Trees' adaptations to the fluctuations of their environment are imprinted in their xylem (Wimmer, 2002) and studying these anatomical variations can provide valuable information on trees'

environment and their adaptation (Schweingruber, 2006). However, identifying and manually measuring a statistically relevant number of anatomical features can be tedious and time consuming, hindering the analyses of pith-to-bark anatomical variability of large numbers of samples. Wood density is determined by chemical and anatomical features of the xylem (Lachenbruch & McCulloh, 2014) and contains thus information on wood anatomy variability.

In Chapter 2, we hypothesised that wood density profiles can be used as proxies for wood anatomy variability. We tried to disentangle the relative contributions to wood density of fibres, vessels and parenchyma for eight tropical tree species. Our results showed that, overall, vessels and parenchyma negatively influence wood density, while fibres are positively related to it. Fibre anatomy (fibre wall thickness – total cell size ratio) impacted wood density the most, corroborating thus the previous studies on that topic (Zieminska *et al.*, 2013).

While the impact of fibre anatomy on wood density is clear, the effect of vessels and parenchyma is less straightforward. In previous studies, little to no correlation between vessels or parenchyma and wood density was reported (Martinez-Cabrera *et al.*, 2009; Zieminska *et al.*, 2013). Not only we showed that wood density – wood anatomy relationships vary between species, but also that vessels or parenchyma can locally strongly influence wood density in certain species. We detected a large intra-individual and inter-species variability in the relationship between wood density and vessels or parenchyma (some portions of a sample even showing opposite relationship between wood density and anatomical features than the whole sample), indicating that their influence on wood density is complex and that interactions between different anatomical features occur. Our study lacks, however, an analysis of the intra-species variability of the wood density – wood anatomy relationship. Since the samples preparation prior to the automated detection and measuring of anatomical features is a time consuming process, a trade-off between the number of individuals per species and the number of species was operated. Yet, a more in-depth exploration of intra-species variability is vital, as proposed

in the Chapter 1. The goal of this study was the development of a new method of studying wood anatomy in order to apply it on larger sample sets of species of interest in the future.

The complexity of the interactions between anatomical features and wood density is a major obstacle in using density profiles for tree-ring boundary identification. While species with ring boundaries characterized by fibre variations and thus density fluctuations (De Ridder *et al.*, 2011) such as *A. mannii* or *S. kamerunensis* can be used in tree-ring analysis by densitometry (Brienen *et al.*, 2016), numerous tropical species with more complex anatomies remain ineligible for those protocols (*M. excelsa*). For example, the alternation of fibres and vessels/parenchyma causes rapidly oscillating density fluctuations, which are difficult to distinguish from density variations at ring boundaries (Worbes, 1995).

In addition to the complexity of wood anatomy – density interactions of several species, wood anatomical studies are hampered by the challenges encountered during sample preparations. In order to reduce uncertainties due to measuring errors, high quality samples and images must be used. More specifically in the case of automated fibre measurements, a high contrast between cell walls and lumina and between different anatomical features is needed. It is challenging to obtain proper thin sections of tropical hardwoods, especially the denser ones. While short cross-sections allow obtaining such quality, they are generally time-consuming to obtain and challenging to stitch together. On the other hand, increasing the length of the sections necessitates an increase of their thickness in order to avoid tearing them, influencing thus subsequent image acquisition. With modern techniques such as Extended Focal Imaging and Multiple Images Alignment (Olympus ©) an image is composed of several superimposed images taken from different heights patched together. However, thicker samples influence the light path and create chromatic aberrations that significantly complicate the automation of the image acquisition and subsequent analysis. To avoid these problems, we worked on polished wood surfaces. This approach allows polishing several cores at the same time and helps overcoming the thickness issue. Yet, some problems related to the colour uniformity of several tissues (dust-filled

vessels, parenchyma and fibres lumina) remain and represent a challenge for the upcoming studies. For the future studies, we advise the use of polished surfaces if there is a need for large numbers of pith-to-bark anatomy images and the use of thin sections of limited size if the quality of the samples and the contrast between different tissues are the number one priority.

Furthermore, recent advances in high-resolution densitometry allow observing the internal structure of objects in 3D with a resolution up to submicron levels (Van den Bulcke *et al.*, 2009; Dierick *et al.*, 2014), making it possible to measure different anatomical features and sidestepping the sample preparation and image acquisition stages. The use of the obtained 3D images for measurements would also prevent a loss of information due to the reduction to a single 1D profile (the pith-to-bark density profile). However, current technical limitations limit the maximal length of the samples that it is possible to process at the highest resolution and limit therefore its application to small portions of the sample.

Despite these persisting issues, our results open new perspectives in tree growth studies. By showing that if wood density variability is due to variations in fibres anatomy, we can analyse their intra-annual density fluctuations from a biological point of view and detect growth-ring distinctness with wood density profiles. While it remains challenging detecting growth-ring boundaries for a plethora of other tropical species, we contributed to improve the knowledge of the anatomical drivers of wood density fluctuations and encourage further efforts in linking wood density profiles to intra-annual xylem features variation as it has the potential to significantly increase the speed of analyses of past and present tree growth. These efforts can be directed towards specifically determined species, on the basis of their relevance to the study (economically important species, biomass hyper-dominant species, endangered species, etc…). With cambial marking and developing fast sampling methods such as micro coring, protocols allowing to link specific climatic events to cambial activity can be elaborated to help reconstructing past growth conditions and study these species reactions to environmental changes. Moreover, X-ray densitometry allows a fast acquisition of increasingly high-resolution pith-

to-bark density profiles and can help rapidly gather large datasets covering large geographic extents for a vast array of trees with different DBH.

Furthermore, our approach of wood anatomy analysis is, to the best of our knowledge, the first of its kind. The automation of detection and measuring of different anatomical features allows a systematic screening of large portions of wood on a pith-to-bark axis, which enables us to study the co-evolution of different anatomical features on the tree level and describe their variability throughout the entire growth ring. Moreover, the relationship between wood density and wood anatomy enables us to derive new metrics characterizing and quantifying the variability of wood anatomical features from wood density profiles. In Chapter 3, we explored new ways of studying tree growth based on the relationship between wood density and wood anatomy described in the second chapter.

- Study of the influence of the seasonality of precipitation on leaf phenology and wood anatomy

Based on our previous findings, we used wood density profiles as proxies for wood anatomy and studied its variability throughout the pith-to-bark axis for 13 common species from the Congo Basin. We derived three descriptors characterizing wood density variability: rhythmicity, regularity and the amplitude of fluctuations. Based on the premise that the anatomical variations that are behind wood density fluctuations are driven by varying cambial activity patterns, which are time dependent, we made the assumption that wood density profiles are an expression of past tree growth.

Significant differences in anatomical variations were detected between evergreen and deciduous trees and between trees in the driest and the wettest sites, in line with previous studies (Moya & Filho, 2009). In parallel, old long-term records of leaf phenology were browsed and leaf shedding patterns compared between two sites. Trees of deciduous species shed their leaves for longer periods and were more synchronous in leaf shedding in the driest site compared to the wettest, corroborating previous studies (Brienen *et al.*, 2016, Nath *et al.*, 2016).

Although the influence of leaf phenology on tree growth and wood formation is known (Borchert, 1999; Borchert *et al.*, 2005; Lisi *et al.*, 2008) and a similar trend was observed in our study, our

experimental design didn't allow to establish a strong leaf phenology – growth relationship at the individual or the species level. Indeed, not only the phenological observations were carried out on different trees than those sampled during our field mission, but a 70-year-long gap separated both studies. However, linking leaf phenology to wood anatomy is essential as it would allow mapping different anatomical features on a temporal axis and estimate trees responses to different climatic events (De Mil, 2017). Recent studies focus on the simultaneous monitoring of leaf and cambial phenology (De Mil, 2017) which might help reducing the knowledge gap in that area and estimate tree adaptation in function of time and environment.

The main goal of this study was to contribute to the estimation of the effects of different environmental conditions on tropical forest dynamics. We showed that evergreen and deciduous species behave differently when water availability decreases. While evergreen species showed a steadier variability of wood density, indicating a more uniform past growth, they were also the most sensitive to the difference of growth conditions between the study sites. This indicates that shifting precipitation patterns might impact evergreen species' growth more. These results can be useful in the framework of the study of the climate change on the forest dynamics. Moreover, differences in leaf phenology induced by variable precipitation patterns observed in our study may impact photosynthetic seasonality of tropical forests and need to be taken in account for modelling gas fluxes and ecosystem responses to climate change (Wu *et al.*, 2016).

However, we must remain cautious while interpreting such results as substituting temporally shifting environmental conditions by geographical gradients only gives an approximation of the studied effects (the rates of the changes are not taken in account, effects of trees' long-term adaptations can be overlooked, other environmental variables can also shift and interact with the studied variables). Moreover, a large intra-species variability of growth descriptors was detected in our study, indicating that other site-dependent micro-climatic variables might strongly influence tree growth (*e.g.* light availability, disturbance history, soil fertility, soil water retention capacity, …) and their effects are

difficult to disentangle from the other variables (temperature, precipitation) *in situ*. We think that an approach focusing on both macro- and micro-climatic variables is needed. We argue in favour of studying large numbers of trees across different environmental gradients and precisely measuring all types of environmental variables in the vicinity of the studied tree in order to construct large databases that allow statistical analyses of the climate-growth relationship.

This study was also a first attempt in using wavelet analysis of wood density profiles. We believe wavelet analysis is a powerful tool that can help unravelling different trends in wood growth, especially when dealing with seasonally recurring patterns. The potential of wavelet analysis has not been fully explored yet but our work shows that it can help deriving different metrics that quantify different aspects of tree growth (*e.g.* rhythmicity, periodicity, amplitude). It is important to remember however that wavelet analysis is designed for studying *temporally* varying signals (Morlet, 1983) and the most usual output is a time-frequency representation of the signal (Schaefli & Zehe, 2009). On the other hand, a wood density profile is a function of the distance and is not a steady temporal representation of the cambial activity (Bouriaud *et al.*, 2005). It is therefore necessary to convert these profiles to time units in order to link them with environmental conditions variations. The aforementioned techniques (micro-coring, cambial wounding) can be helpful in that effort.

Finally, it is important to keep in mind that this approach is subjected to other persisting issues of tropical tree-ring studies. Wedging rings are a common problem encountered in tropical dendrochronology (Worbes, 2002). As a consequence, there is a certain degree of intra-tree variability between different axes. It is not yet clear how to deal with this variability. In this study, we averaged the descriptors calculated for each core at the tree level (three or four cores per tree). However, other approaches such as using only the longest core or a larger number of cores per tree may be elaborated. We advocate in favour of further research to promote the use of wood cores despite intra-tree variability as cores are much less destructive than tree disks and are crucial to build large datasets as proposed above.

General conclusion and recommendations for further studies

In this dissertation, we aimed to improve our understanding of the wood density – wood anatomy relationship and to explore new methods of studying tree growth. Dendroclimatology is among the most common tools to study climate – growth relationship and has a long history of application in the temperate latitudes. However, in the tropics, subtle inter- and intra-annual climatic variations increase the complexity of wood anatomy and the variability of growth-ring distinctness. This variability is poorly acknowledged and there is currently a lack of databases featuring this information. We argue in favour of the creation of such databases that would allow studying the variation of wood anatomy and density as well as growth-ring distinctness across sites with different environmental conditions.

Furthermore, the study of intra-annual variability of wood anatomy is emerging as a powerful tool for studying tree growth in the tropics. The increasing computational power coupled to improving imaging techniques allow scientists to gather and process larger numbers of wood samples from the tropics. X-ray computed tomography densitometry allows measuring wood density (and even xylem features on small portions of wood) for large numbers of wood cores while automated measuring of anatomical features allows analysing intra-annual xylem features from pith to bark. We believe that these two techniques have the potential to significantly increase the precision and the speed of wood anatomy analysis.

In this study, we showed that the wood density – anatomy relationship is species dependent and that further research is required to use wood density profiles as proxies for wood anatomy in many tropical species. We encourage thus a more thorough exploration of this relationship for different species of interest such as biomass hyper-dominant species. Developing species-specific allometric equations linking wood anatomy to wood density will help understanding these species' growth more precisely and better predict the influence of climate change on tropical forests.

To link the observed anatomy changes to the varying environmental conditions, wood anatomical features need to be mapped on a time axis. Leaf and cambium phenology monitoring can help

correlating tree growth to climatic events occurring during the study. We also used a geographical approach, studying trees responses in function of the variation of the environmental conditions along a geographic gradient. This approach, although showing interesting trends in tree growth, also highlighted the importance of site-dependent micro-environmental conditions. For further studies, we strongly encourage a thorough description of the immediate environments of the trees in order to maximize the statistical power of studies.

Our findings also show that it is possible to quantify different aspects of wood growth in order to compare trees but also monitor their evolution throughout a tree's life. The wavelet analysis allowed us deriving different descriptors (rhythmicity, the regularity and the intensity of wood density fluctuations) and compare them between individuals, species and study sites. We believe that the wavelet analysis, thanks to its broad domain of applications and its analytical power for cyclical signals, has the potential to become a powerful tool in studying different processes related to climate and tree growth.

Finally, our efforts helped detecting several differences in tree growth between different study sites. We showed that evergreen species adapted differently to longer dry seasons than deciduous ones. The phenology of trees adapted to longer dry seasons showed an increased period of lead shedding and inter-trees synchronicity of phenological events. These trends need to be explored further as they can significantly impact tropical forests in the context of the climate change.

Take home message

The main results obtained in the present work are the following:

- There is an important intra- and inter-species variability of growth-ring boundaries distinctness in tropical hardwoods that should be acknowledged while constructing global databases;
- The wood density – wood anatomy relationship is species-dependent and locally highly variable: different anatomical features can associate with each other and exert varying effects on wood density depending on the species and the surrounding features;

- High resolution wood density profiles can be used as proxies to detect and study certain types of anatomical features variations: higher density is mostly driven by flattened fibres and/or thickened fibre walls;
- Wavelet analysis are a powerful tool for analysing wood density variability: they allow quantifying variability patterns with a plethora of different metrics and analysing large numbers of wood density profiles with a standardized method;
- Evergreen and deciduous species show different levels of adaptation to varying precipitation conditions: wood density fluctuations of evergreen species increase in Luki indicating that precipitation seasonality might impact more their cambial activity;
- Site-dependent micro-environmental conditions play an important role in tree growth and need to be thoroughly described in future tree growth studies.

These results provide new insights and promising tools for future tree studies. In particular, the importance of wood density profiles in the analysis of wood anatomy and its intra-annual variations is highlighted. We also show that new metrics quantifying wood growth can be derived with wavelet analysis.

In the context of climate change, we believe our findings will help developing new protocols for studying climate – tree growth relationship and assessing the future of tropical forest.

References

Akachuku AE. **1985**. Intra-annual variation in wood density in *Gmelina arborea* from X-ray densitometry and its relationship with rainfall. *Tree-Ring Bulletin* **45**: 43–53.

Allen C, Macalady A, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears D, Hogg E *et al.* 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* **259**: 660-684.

Alvim P. 1964. Tree growth periodicity in tropical climates. In: Zimmerman M, ed. *The formation of wood in forest trees*., NY, USA: Academic Press. 479–495.

Anderegg WR, Berry J A, Field CB. 2012. Linking definitions, mechanisms, and modelling of drought-induced tree death. *Trends in plant science* **17(12):** 693-700.

Arganda-Carreras I, Kaynig V, Rueden C, Eliceiri KW, Schindelin J, Cardona A, Seung HS. **2017**. Trainable Weka Segmentation: A machine learning tool for microscopy pixel classification. *Bioinformatics* **33**: 2424–2426.

Aragão LEOC, Poulter B, Barlow JB, Anderson LO, Malhi Y, Saatchi S, Phillips OL, Gloor E. 2014. Environmental change and the carbon balance of Amazonian forests. *Biological Reviews* **89:** 913–931.

Baas P. 1982. Leeuwenhoek's contributions to wood anatomy and his ideas on sap transport in plants. pp. 79—107 in: Palm LC & Snelders HAM (eds), Antoni van Leeuwenhoek 1632—1982. Rodopi, Amsterdam.

Baas P, Vetter RE. 1989. Growth rings in tropical trees. *IAWA bull (Special Issue)* **10:** 95-174.

Babst F, Bouriaud O, Papale D, Gielen B, Janssens IA, Nikinmaa E, Ibrom A, Wu J, Bernhofer C, Köstner B, Grünwald T, Seufert G, Ciais P, Frank D. 2014. Above-ground woody carbon sequestration measured from tree rings is coherent with net ecosystem productivity at five eddy-covariance sites. *New Phytologist* **201**: 1289-1303.

Badel E, Ewers FW, Cochard H, Telewski FW. 2015. Acclimation of mechanical and hydraulic functions in trees: impact of the thigmomorphogenetic process. *Frontiers in plant science* **6**: 266.

Baker TR, Phillips OL, Malhi Y, Almeida S, Arroyo L, Di Fiore A, Erwin T, Killeen TJ, Laurance SG, Laurance WF, *et al.* 2004. Variation in wood density determines spatial patterns in Amazonian forest biomass. *Global Change Biology* **10**: 545–562.

Baker PJ, Bunyavejchewin S. 2006. Suppression, release and canopy recruitment in five tree species from a seasonal tropical forest in western Thailand. *Journal of Tropical Ecology* **22:** 521–529.

Baldeck C, Asner G, Martin R, Anderson C, Knapp D, Kellner J, Wright S. 2015. Operational Tree Species Mapping in a Diverse Tropical Forest with Airborne Imaging Spectroscopy. *PLOS ONE* **10**: e0118403.

Bastin JF, Barbier N, Couteron P, Adams B, Shapiro A, Bogaert J, De Cannière C. 2014. Aboveground biomass mapping of African forest mosaics using canopy texture analysis: toward a regional approach. *Ecological Applications* **24**: 1984-2001.

Bastin JF, Barbier N, Réjou-Méchain M, Fayolle A, Gourlet-Fleury S, Maniatis D, ..., Couteron P. 2015a. Seeing Central African forests through their largest trees. *Scientific Reports* **5:** 13156.

Bastin JF, Fayolle A, Tarelkin Y, Van Den Bulcke J, De Haulleville T, Mortier F, Beeckman H, Van Acker J, Serckx A, Bogaert J, De Cannière C. 2015. Wood specific gravity variations and biomass of central African tree species: The simple choice of the outer wood. *PLoS ONE* **10**: 1–16.

Bastin JF, Berrahmouni N, Grainger A, Maniatis D, Mollicone D, Moore R, ..., Castro R. 2017. The extent of forest in dryland biomes. *Science* **356(6338):** 635-638.

Batschelet E. 1981. *Circular statistics in biology*. London: Academic Press.

Battipaglia G, Campelo F, Vieira J, Grabner M, Micco V De, Nabais C, Cherubini P, Carrer M, Bräuning A, Simon J. **2016**. Structure and function of intra–annual density fluctuations: mind the gaps. *Frontiers in plant science* **7:** 595.

Beeckman H. **2016**. Wood anatomy and trait-based ecology. *IAWA Journal* **37**: 127–151.

Bellassen V, Luyssaert S. 2014. Managing forests in uncertain times. *Nature* **506:** 153-155.

Benedet F, Doucet J, Fayolle A, Gourlet-Fleury S, Vincke D. Cofortraits, African plant traits information database. version 1.0. http://coforchange.cirad.fr/african_plant_trait [accessed 28 September 2016].

Bergsten U, Lindeberg J, Rindby A, Evans R. **2001**. Batch measurements of wood density on intact or prepared drill cores using x-ray microdensitometry. *Wood Science and Technology* **35**: 435–452.

Biudes M, Vourlitis G, Machado N, de Arruda P, Neves G, de Almeida Lobo F, Neale C, de Souza Nogueira J. 2015. Patterns of energy exchange for tropical ecosystems across a climate gradient in Mato Grosso, Brazil. *Agricultural and Forest Meteorology* **202**: 112-124.

Björklund JA, Gunnarson BE, Seftigen K, Esper J, Linderholm HW. **2014**. Blue intensity and density from northern Fennoscandian tree rings, exploring the potential to improve summer temperature reconstructions with earlywood information. *Climate of the Past* **10**: 877–885.

Björklund J, Seftigen K, Schweingruber F, Fonti P, von Arx G, Bryukhanova MV, Cuny HE, Carrer M, Castagneri D, Frank DC. **2017**. Cell size and wall dimensions drive distinct variability of earlywood and latewood density in Northern Hemisphere conifers. *New Phytologist* **216(3):** 728-740.

Bonan G. 2008. Forests and Climate Change: Forcings, Feedbacks, and the Climate Benefits of Forests. *Science* **320**: 1444-1449.

Borchert R. 1994. Soil and stem water storage determine phenology and distribution of tropical dry forest trees. *Ecology* **75:** 1437–1449.

Borchert R. 1999. Climatic Periodicity, Phenology, and Cambium Activity in Tropical Dry Forest Trees. *IAWA Journal* **20**: 239-247.

Borchert R, Calle Z, Strahler A, Baertschi A, Magill R, Broadhead J, Kamau J, Njoroge J, Muthuri C. 2015. Insolation and photoperiodic control of tree development near the equator. *New Phytologist* **205**: 7-13.

Bouriaud O, Leban JM, Bert D, Deleuze C. **2005**. Intra-annual variations in climate influence growth and wood density of Norway spruce. *Tree physiology* **25**: 651–60.

Brando PM, Nepstad DC, Davidson EA, Trumbore SE, Ray D, Camargo P. 2008. Drought effects on litterfall, wood production and belowground carbon cycling in an Amazon forest: results of a throughfall reduction experiment. *Philosophical Transactions of the Royal Society B: Biological Sciences* **363(1498):** 1839-1848.

Bräuning A, De Ridder M, Zafirov N, García-González I, Dimitrov DP, Gärtner H. 2016. Tree-ring features – indicators of extreme event impacts. *IAWA Journal* **37(2):** 206-231.

Brienen RJW, Zuidema PA. 2005. Relating tree growth to rainfall in Bolivian rain forests: a test for six species using tree ring analysis. *Oecologia* **146(1):** 1-12.

Brienen RJW, Zuidema PA. 2006. Lifetime growth patterns and ages of Bolivian rain forest trees obtained by tree ring analysis. *Journal of Ecology* **94(2):** 481-493.

Brienen RJW, Phillips OL, Feldpausch TR, Gloor E, Baker TR, Lloyd J. 2015. Long-term decline of the Amazon carbon sink. *Nature* **519:** 344–348.

Brienen RJW, Schöngart J, Zuidema PA. **2016**. Tree Rings in the Tropics: Insights into the Ecology and Climate Sensitivity of Tropical Trees. In: Goldstein G, Santiago LS, eds. Tropical Tree Physiology: Adaptations and Responses in a Changing Environment. Cham: Springer International Publishing, 439–461.

Briffa KR. **1998**. Trees tell of past climates: but are they speaking less clearly today? *Philosophical Transactions of the Royal Society B: Biological Sciences* **353**: 65–73.

Briffa K, Melvin T. 2011. A closer look at regional curve standardization of tree-ring records: justification of the need, a warning of some pitfalls, and suggested improvements in its application. In: *Dendroclimatology*. Springer Netherlands. 113-145.

Brodribb T. 2017. Progressing from 'functional' to mechanistic traits. *New Phytologist* **215**: 9-11.

Bullmore E, Fadili J, Breakspear M, Salvador R, Suckling J, Brammer M. 2003. Wavelets and statistical analysis of functional magnetic resonance images of the human brain. *Statistical methods in medical research*, **12(5)**, 375-399.

Calfapietra C, Ainsworth EA, Beier C, De Angelis P, Ellsworth DS, Godbold DL, ..., Ceulemans R. 2010. Challenges in elevated CO2 experiments on forests. *Trends in plant science* **15(1):** 5-10.

Callado C, Barros C, Costa C, da Silva Neto S, Scarano F. 2001. Anatomical features of growth rings in flood-prone trees of the Atlantic rain forest in Rio De Janeiro, Brazil. *IAWA Journal* **22**: 29-42.

Carlquist S. 1988. *Comparative wood anatomy*. Berlin: Springer-Verlag.

Carlquist S. 2001. Comparative Wood Anatomy: Systematic Ecological, and Evolutionary Aspects of Dicotyledon Wood. Springer-Verlag, Berlin, 448.

Cassart B, Basia AA, Titeux H, Andivia E, Ponette Q. 2016. Contrasting patterns of carbon sequestration between *Gilbertiodendron dewevrei* monodominant forests and *Scorodophloeus zenkeri* mixed forests in the Central Congo basin. *Plant and Soil* **414**: 309-326.

Cassart B. 2018. Effect of community functional trait composition on soil organic carbon dynamics between Gilbertiodendron dewevrei monodominant forests and Scorodophloeus zenkeri mixed forests in the Central Congo basin (Doctoral dissertation, UCL-Université Catholique de Louvain).

Cavaleri MA, Reed SC, Smith WK, Wood TE. **2015**. Urgent need for warming experiments in tropical forests. *Global Change Biology* **21**: 2111–2121.

Chave J, Andalo C, Brown S, Cairns MA, Chambers JQ, Eamus D, Fölster H, Fromard F, Higuchi N, Kira T, Lescure JP, Nelson BW, Ogawa H, Puig H, Riéra B, Yamakura T. 2005. Tree allometry and improved estimation of carbon stocks and balance in tropical forests. *Oecologia* **145(1):** 87–99.

Chave J, Condit R, Muller-Landau H, Thomas S, Ashton P, Bunyavejchewin S, Co L, Dattaraja H, Davies S, Esufali S, …, Losos EC. 2008. Assessing Evidence for a Pervasive Alteration in Tropical Tree Communities. *PLoS Biology* **6**: e45.

Chave J, Coomes D, Jansen S, Lewis SL, Swenson NG, Zanne AE. **2009**. Towards a worldwide wood economics spectrum. *Ecology Letters* **12**: 351–366.

Chave J, Réjou-Méchain M, Búrquez A, Chidumayo E, Colgan MS, Delitti WB, ..., Henry M. 2014. Improved allometric models to estimate the aboveground biomass of tropical trees. *Global change biology* **20(10):** 3177-3190.

Cherubini P, Humbel T, Beeckman H, Gärtner H, Mannes D, Pearson C, Schoch W, Tognetti R, Lev-Yadun S. 2013. Olive tree-ring problematic dating: a comparative analysis on Santorini (Greece). *PloS one* **8(1):** e54730.

Choat B, Sack L, Holbrook M. 2007. Diversity of hydraulic traits in nine Cordia species growing in tropical forests with contrasting precipitation. *New Phytologist* **175:** 686–698.

Clark DA. 2007. Detecting tropical forests' responses to global climatic and atmospheric change: current challenges and a way forward. *Biotropica* **39:** 4–19

Clark JS, Bell DM, Hersh MH, Nichols L. 2011. Climate change vulnerability of forest biodiversity: climate and competition tracking of demographic rates. *Global Change Biology* **17(5):** 1834-1849.

Cleveland CC, Taylor P, Chadwick KD, Dahlin K, Malhi Y, Smith WK, Sullivan BW, Wieder WR, Townsend AR. 2015. A comparison of plot-based, satellite, and Earth System model estimates of tropical net primary production. *Global Biogeochemical Cycles* **29(5):** 626-644.

Connell JH, Lowman MD. 1989. Low-diversity tropical rain forests: some possible mechanisms for their existence. *The American Naturalist* **134(1)**: 88-119.

Corlett RT. 2011. Impacts of warming on tropical lowland rainforests. *Trends in Ecology and Evolution* **26:** 606–613

Cornelissen JHC, Lavorel S, Garnier E, Diaz S, Buchmann N, Gurvich DE, Reich PB, ter Steege H, Morgan HD, van der Heijden MGA, Poorter H, Pausas JG. 2003. A handbook of protocols for standardized and easy measurement of plant functional traits worldwide. *Australian journal of Botany* **51:** 335--380.

Couralet C, Sterck F, Sass-Klaassen U, Van Acker J, Beeckman H. 2010. Species-Specific Growth Responses to Climate Variations in Understory Trees of a Central African Rain Forest. *Biotropica* **42**: 503-511.

Couralet C, Van den Bulcke J, Ngoma L, Van Acker J, Beeckman H. 2013. Phenology in functional groups of central African rainforest trees. *Journal of Tropical Forest Science*, 361-374.

Dadzie PK, Amoah M, Frimpong-Mensah K, Shi SQ. **2016**. Comparison of density and selected microscopic characteristics of stem and branch wood of two commercial trees in Ghana. *Wood Science and Technology* **50**: 91–104.

Davies S, Ashton P. 1999. Phenology and Fecundity in 11 Sympatric Pioneer Species of Macaranga (Euphorbiaceae) in Borneo. *American Journal of Botany* **86**: 1786-1795.

De Frenne P, Verheyen K. 2016. Weather stations lack forest data. *Science* **351**: 234-234.

De Micco V, Campelo F, Cherubini P, Battipaglia G, Bräuning A, Grabner M, De Luis M. 2016. Intra-annual density fluctuations in tree rings: how, when, where, and why? *IAWA Journal* **37**: 232-259.

De Mil T, Vannoppen A, Beeckman H, Van Acker J, Van den Bulcke J. **2016**. A field-to-desktop toolchain for X-ray CT densitometry enables tree ring analysis. *Annals of Botany* **117**: 1187–1196.

De Mil T, Angoboy Ilondea B, Maginet S, Duvillier J, Van Acker J, Beeckman H, den Bulcke J. **2017**. Cambial activity in the understory of the Mayombe forest, DR Congo. *Trees – Structure and function* **31**: 49–61.

De Mil T, Beeckman H, Van Acker J, Van den Bulcke J. 2017. Intra-annual to multi-decadal xylem traits in a tropical moist semi-deciduous forest of Central Africa. Gent: UGent.

De Ridder M, Van den Bulcke J, Vansteenkiste D, Van Loo D, Dierick M, Masschaele B, De Witte Y, Mannes D, Lehmann E, Beeckman H, *et al.* 2011. High-resolution proxies for wood density variations in *Terminalia superba*. *Annals of botany* **107**: 293–302.

De Ridder M, Trouet V, Van den Bulcke J, Hubau W, Van Acker J, Beeckman H. 2013. A tree-ring based comparison of *Terminalia superba* climate–growth relationships in West and Central Africa. *Trees* **27**: 1225-1238.

Denman KL *et al.*, in Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, S. Solomon et al., Eds. (Cambridge Univ. Press, Cambridge, 2007) pp. 499–587.

Dierick M, Van Loo D, Masschaele B, Van den Bulcke J, Van Acker J, Cnudde V, Van Hoorebeke L. **2014**. Recent micro-CT scanner developments at UGCT. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* **324**: 35–40.

Dierick M, Masschaele B, Van Hoorebeke L. **2004**. Octopus, a fast and user-friendly tomographic reconstruction package developed in LabView®. *Measurement Science and Technology* **15**: 1366–1370.

Dong S, Davies S, Ashton P, Bunyavejchewin S, Supardi M, Kassim A, Tan S, Moorcroft P. 2012. Variability in solar radiation and temperature explains observed patterns and trends in tree growth rates across four tropical forests. *Proceedings of the Royal Society B: Biological Sciences* **279**: 3923-3931.

Donis C. 1948. Essai D'économie Forestiere au Mayumbe. INEAC, Bruxelles, Belgium.

Dlugokencky E, Tans P. 2018. Recent CO2, NOAA, ESRS. www.esrl.noaa.gov/gmd/ccgg/trends/global.html.

Dunne K, Willmott C. 2000. Global Distribution of Plant-Extractable Water Capacity of Soil (Dunne). ORNL DAAC, Oak Ridge, Tennessee, USA. http://dx.doi.org/10.3334/ORNLDAAC/545. [accessed 15 March 2017].

Enquist BJ, West GB, Charnov EL, BrownJH. 1999. Allometric scaling of production and life-history variation in vascular plants. *Nature* **401:** 907–911.

Fahn A. 1962. Xylem structure and the annual rhythm of cambial activity in woody species of the East Mediterranean regions. *News Bull. Int. Ass. Wood Anat.* **(1):** 2-6.

Fan ZX, Zhang SB, Hao GY, Ferry Slik JW, Cao KF. 2012. Hydraulic conductivity traits predict growth rates and adult stature of 40 Asian tropical tree species better than wood density. *Journal of Ecology* **100:** 732–741.

FAO. 1979. Management properties of Ferralsols. FAO Soils Bulletin 23. FAO, Rome.

FAO. 2006. Global forest resources assessment 2005: progress towards sustainable forest Management

FAO. 2007. *Digital soil map of the world*. FAO, Rome.

FAO. 2016. *State of the World's forests*. FAO. Rome.

Farge M. 1992. Wavelet transforms and their applications to turbulence. *Annual review of fluid mechanics*, **24(1)**, 395-458.

Fayolle A, Picard N, Doucet JL, Swaine M, Bayol N, Bénédet F, Gourlet-Fleury S. 2014. A new insight in the structure, composition and functioning of central African moist forests. *Forest Ecology and Management* **329:** 195–205.

Fichtler E, Worbes M. **2012**. Wood anatomical variables in tropical trees and their relation to site conditions and individual tree morphology. *IAWA Journal* **33**: 119–140.

Fick SE, Hijmans RJ. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology* **37(12)**: 4302-4315.

Fiorio C, Gustedt J. 1996. Two linear time union-find strategies for image processing. *Theoretical Computer Science **154*(2)**: 165-181.

Fisher JB, Sikka M, Sitch S, Ciais P, Poulter B, Galbraith D, Lee J, Huntingford C, Viovy N, Zeng N, Ahlström A, Lomas MR, Levy PE, Frankenberg C, Saatchi S, Malhi Y. 2013. African tropical rainforest net carbon dioxide fluxes in the twentieth century. *Philosophical Transactions of the Royal Society B* **368(1625):** 20120376.

Foley J, DeFries R, Asner G, Barford C, Bonan G, Carpenter S, Chapin S, Coe M, Daily G, Gibbs H, Helkowski J, Holloway T, Howard E, Kucharik C, Monfreda C, Patz J, Prentice I, Ramankutty N, Snyder P. 2005. Global consequences of land use. *Science* **309:** 570– 574.

Fonti P, Von Arx G, García-González I, Eilmann B, Sass-Klaassen U, Gärtner H, Eckstein D. 2010. Studying global change through investigation of the plastic responses of xylem anatomy in tree rings. *New Phytologist* **185**: 42-53.

Francis JE, Hill RS. 1996. Fossil plants from the Pliocene Sirius Group, Transantarctic Mountains: evidence for climate from growth rings and fossil leaves. *Palaios* 389-396.

Fritts HC. 1976. Tree rings and climate. Academic Press, London

Gartner B. 1995. *Plant stems: physiology and functional morphology*. Academic Press.

Gärtner H, Nievergelt D. 2010. The core-microtome: a new tool for surface preparation on cores and time series analysis of varying cell parameters. *Dendrochronologia* ***28*****(2)**: 85-92.

GEA. 2006. Energy resources and potentials. Pages 425–512 Global Energy Assessment—Toward a Sustainable Future. Cambridge University Press, Cambridge, United Kingdom, and New York, NY, USA.

Gebrekirstos A, Bräuning A, Sass-Klassen U, Mbow C. 2014. Opportunities and applications of dendrochronology in Africa. *Current Opinion in Environmental Sustainability* **6:** 48–53.

Gilbert SG. 1940. Evolutionary Significance of Ring Porosity in Wood Angiosperms. Botanical Gazette **102:** 105-120.

Gilson P. 1956. Map of soils and vegetation of the Belgian Congo and Ruanda-Urundi. Explanatory note. 6. Yangambi. Survey 2: Yangambi. Publications de l'Institut National pour l'Etude Agronomique du Congo Belge.

Gotsch S, Geiger E, Franco A, Goldstein G,Meinzer F, HoffmannW. 2010. Allocation to leaf area and sapwood area affectswater relations of co-occurring savanna and forest trees. *Oecologia* **163:** 291–301.

Gourlay ID. 1995. The definition of seasonal growth zones in some African Acacia species-a review. IAWA Journal, **16:** 353-359.

Gourlay ID. 1995. Growth Ring Characteristics of Some African Acacia Species. Journal of Tropical Ecology, **11(1):** 121-140.

Greenwood S, Ruiz-Benito P, Martínez-Vilalta J, Lloret F, Kitzberger T, Allen C, Fensham R, Laughlin D, Kattge J, Bönisch G, Kraft NJB, Jump AS. 2017. Tree mortality across biomes is promoted by drought intensity, lower wood density and higher specific leaf area. *Ecology Letters* **20**: 539-553.

Groenendijk P, Sass-Klaassen U, Bongers F, Zuidema P. 2014. Potential of tree-ring analysis in a wet tropical forest: A case study on 22 commercial tree species in Central Africa. *Forest Ecology and Management* **323**: 65-78.

Groombridge B, Jenkins MD. 2003. World atlas of biodiversity. Berkeley, CA: University of California Press.

Guilley E, Mothe F, Nepveu G. **2002**. A procedure based on conditional probabilities to estimate proportions and densities of tissues from X-ray images of Quercus petraea samples. *IAWA Journal* **23**: 235–252.

Haralick R, Shanmugam K, Dinstein I. 1973. Textural Features for Image Classification. *IEEE Transactions on Systems, Man, and Cybernetics* **6:** 610-621.

Hietz P, Valencia R, Joseph Wright S. **2013**. Strong radial variation in wood density follows a uniform pattern in two neotropical rain forests. *Functional Ecology* **27**: 684–692.

Hijmans R, Cameron S, Parra J, Jones P, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* **25**: 1965-1978.

Hoadley RB. 1990. *Identifying wood: accurate results with simple tools*. Taunton Press.

Holtum JAM, Winter K. 2010. Elevated [CO2] and forest vegetation: More a water issue than a carbon issue? *Functional Plant Biology* **37:** 694-702.

Huang JG, Bergeron Y, Denneler B, Berninger F, Tardif J. 2007. Response of forest trees to increased atmospheric CO 2. *Critical Reviews in Plant Sciences* **26(5-6):** 265-283.

Hubau W, Van den Bulcke J, Van Acker J, Beeckman H. 2015. Charcoal-inferred Holocene fire and vegetation history linked to drought periods in the Democratic Republic of Congo. *Global change biology* **21(6):** 2296--2308.

Hudson I, Keatley M, Kang I. 2011. Wavelet characterization of eucalypt flowering and the influence of climate. *Environmental and Ecological Statistics* **18(3)**: 513-533.

IAWA Committee. 1989. IAWA list of microscopic features for hardwood identification. *IAWA Bulletin* **10:** 219-332.

IPCC. 2013. Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK *et al*, eds, *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge, UK and New York, NY, USA: Cambridge University Press.

IPCC. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Jacobsen AL, Agenbag L, Esler KJ, Pratt RB, Ewers FW, Davis SD. 2007. Xylem density, biomechanics and anatomical traits correlate with water stress in 17 evergreen shrub species of the Mediterranean-type climate region of South Africa. *Journal of Ecology* **95:** 171–183.

James R, Washington R, Rowell D. 2013. Implications of global warming for the climate of African rainforests. *Philosophical Transactions of the Royal Society B: Biological Sciences* **368**: 20120298-20120298.

Joos F, Spahni R. 2008. Rates of change in natural and anthropogenic radiative forcing over the past 20,000 years. *Proceedings of the National Academy of Sciences of the United States of America* **105:** 1425–30.

Joos F, Roth R, Fuglestvedt JS, Peters GP, Enting IG, Bloh WV, ..., Weaver AJ. 2013. Carbon dioxide and climate impulse response functions for the computation of greenhouse gas metrics: a multi-model analysis. *Atmospheric Chemistry and Physics* **13(5):** 2793-2825.

Kearsley E, de Haulleville T, Hufkens K, Kidimbu A, Toirambe B, Baert G, Huygens D, Kebede Y, Defourny P, Bogaert J, Beeckman H, Steppe K, Boeckx P, Verbeeck H. 2013. Conventional tree height–diameter relationships significantly overestimate aboveground carbon stocks in the Central Congo Basin. *Nature Communications* **4:** 2269.

Keenan TF, Hollinger DY, Bohrer G, Dragoni D, Munger JW, Schmid HP, Richardson AD. 2013. Increase in forest water-use efficiency as atmospheric carbon dioxide concentrations rise. *Nature* **499(7458):** 324.

Kennel P, Subsol G, Guéroult M, Guéroult M, Borianne P. 2010. Automatic identification of cell files in light microscopic images of conifer wood. In *Image Processing Theory Tools and Applications (IPTA), 2010 2nd International Conference on* 98-103. IEEE.

Koch M, Marković D. 2007. Evidences for climate change in Germany over the 20th century from the stochastic analysis of hydro-meteorological time-series. In *MODSIM 2007 International Congress on Modelling and Simulation, Christchurch, New Zealand.*

Köhler P, Knorr G, Buiron D, Lourantou A, Chappellaz J. 2011. Abrupt rise in atmospheric CO2 at the onset of the Bølling/Allerød: in-situ ice core data versus true atmospheric signal. *Climate of the Past* **7(2):** 473-486.

Körner C. 2006. Plant CO2 responses: an issue of definition, time and resource supply. *New Phytologist* **172:** 393–411.

Körner C. 2009. Responses of humid tropical trees to rising CO2. *Annual Review of Ecology, Evolution, and Systematics* **40:** 61-79.

Körner C, Basler D. **2010**. Phenology under global warming. *Science* **327**: 1461–1462.

Lachenbruch B, Mcculloh KA. **2014**. Traits, properties, and performance: How woody plants combine hydraulic and mechanical functions in a cell, tissue, or whole plant. *New Phytologist* **204**: 747–764.

Larson PR. 2012. *The vascular cambium: development and structure*. Springer Science & Business Media.

Lau K, Weng H. 1995. Climate signal detection using wavelet transform: How to make a time series sing. *Bulletin of the American Meteorological Society* **76(12)**: 2391-2402.

Laurance WF, Oliveira AA, Laurance SG, Condit R, Nascimento HE, Sanchez-Thorin AC, Lovejoy TE, Andrade A, D'Angelo S, Ribiero JE, Dick, C. W. 2004. Pervasive alteration of tree communities in undisturbed Amazonian forests. *Nature* **428(6979):** 171.

Lawrence A, Phillips OL, Ismodes AR, Lopez M, Rose S, Wood D, Farfan AJ. 2005. Local values for harvested forest plants in Madre de Dios, Peru: towards a more contextualised interpretation of quantitative ethnobotanical data. *Biodiversity and Conservation* **14:** 45–79.

Le Quéré CL, Andrew RM, Friedlingstein P, Sitch S, Pongratz J, Manning AC, ..., Boden TA. 2018. Global Carbon Budget 2017. *Earth System Science Data* **10(1)**: 405-448.

Lebrun J, Gilbert G. 1954. Une Classification Ecologique des Forêts du Congo. INEAC, Série Scientifique No. 63, Bruxelles, Belgique.

Lens F, Sperry JS, Christman MA, Choat B, Rabaey D, Jansen S. 2011. Testing hypotheses that link wood anatomy to cavitation resistance and hydraulic conductivity in the genus Acer. *New Phytologist* **190:** 709–723.

Lewis SL, Phillips OL, Baker TR, Lloyd J, Malhi Y, Almeida S, ..., Terborgh J. 2004. Concerted changes in tropical forest structure and dynamics: evidence from 50 South American long-term plots. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **359(1443)**: 421-436.

Lewis SL. 2006. Tropical forests and the changing earth system. *Philosophical Transactions of the Royal Society B: Biological Sciences* **361(1465):** 195-210.

Lewis SL, Lloyd J, Sitch S, Mitchard E, Laurance W. 2009. Changing Ecology of Tropical Forests: Evidence and Drivers. *Annual Review of Ecology, Evolution, and Systematics* **40**: 529-549.

Lewis SL, Brando PM, Phillips OL, van der Heijden GMF, Nepstad D. 2011. The 2010 Amazon drought. *Science* **331:** 554.

Lisi C, Roig F, Voigt A, Maria V, Tomazello Fo M, Ferreira-Fedele L, Botosso P. 2008. Tree-Ring Formation, Radial Increment Periodicity, and Phenology of Tree Species from a Seasonal Semi-Deciduous Forest in Southeast Brazil. *IAWA Journal* **29**: 189-207.

Lloyd J, Farquhar GD. 2008. Effects of rising temperatures and [CO2] on the physiology of tropical forest trees. *Philosophical Transactions of the Royal Society B: Biological Sciences* **363(1498):** 1811-1817.

Lu X, Liu R, Liu J, Liang S. 2007. Removal of noise by wavelet method to generate high quality temporal data of terrestrial MODIS products. *Photogrammetric Engineering & Remote Sensing* **73(10)**: 1129-1139.

Lukac M, Calfapietra C, Lagomarsino A, Loreto F. 2010. Global climate change and tree nutrition: effects of elevated CO2 and temperature. *Tree physiology* **30(9):** 1209-1220.

Malhi Y, Grace J. 2000. Tropical forests and atmospheric carbon dioxide. *Trends in Ecology & Evolution* **15:** 332–337.

Malhi Y, Baker TR, Phillips OL, Almeida S, Alvarez E, Arroyo L, Chave J, Czimczik CI, Di Fiore A, ..., Lloyd J. 2004. The above-ground coarse wood productivity of 104 Neotropical forest plots. *Global Change Biology* **10(5):** 563-591.

Malhi Y, Wright J. 2004. Spatial patterns and recent trends in the climate of tropical rainforest regions. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences* **359:** 311–329.

Malhi Y, Roberts J, Betts R, Killen T, Li W, Nobre C. 2008. Climate change, deforestation, and the fate of the Amazon. *Science* **319:** 169-172.

Malhi Y, Adu-Bredu S, Asare RA, Lewis SL, Mayaux P. 2013. African rainforests: past, present and future. *Phil. Trans. R. Soc. B* **368(1625)**: 20120312.

Marcati C, Oliveira J, Machado S. 2006. Growth rings in cerrado woody species: occurrence and anatomical markers. *Biota Neotropica* **6**.

Mariaux A. **1967**. Les cernes dans les bois tropicaux africains, nature et périodicité: peuvent-ils révéler l'âge des arbres? *Bois et Forêts des Tropiques* **113**: 3–14.

Mariaux A, Bossanyi I. 2016. Nature and periodicity of growth rings in African timber: can they be used to determine the age of trees? *Bois et Forêts des Tropiques* **327**: 51-76.

Martínez-Cabrera HI, Jones CS, Espino S, Jochen Schenk H. **2009**. Wood anatomy and wood density in shrubs: Responses to varying aridity along transcontinental transects. *American Journal of Botany* **96**: 1388–1398.

McBride JR. 1983. Analysis of tree rings and fire scars to establish fire history. *Tree-Ring Bulletin* **43:** 51-67.

Milton K. 1991. Leaf Change and Fruit Production in Six Neotropical Moraceae Species. *The Journal of Ecology* **79**: 1-26.

Moreau I. 2016. *Evidencing land cover dynamics and tropical forest seasonality: the benefit of 13 years of daily global observation (SPOT-VEGETATION).* PhD thesis, Université Catholique de Louvain, Belgium.

Morisette JT, Richardson AD, Knapp AK, Fisher JI, Graham EA, Abatzoglou J, Wilson BE, Breshears DD, Henebry GM, Hanes JM, Liang L. 2009. Tracking the rhythm of the seasons in the face of global

change: phenological research in the 21st century. *Frontiers in Ecology and the Environment* **7(5):** 253–260.

Morlet J. 1983. Sampling theory and wave propagation. *Issues in Acoustic signal/Image processing and recognition*, **1**: 233-261.

Morton D, Nagol J, Carabajal C, Rosette J, Palace M, Cook B, Vermote E, Harding D, North P. 2014. Amazon forests maintain consistent canopy structure and greenness during the dry season. *Nature* **506**: 221-224.

Moura Y, Galvão L, dos Santos J, Roberts D, Breunig F. 2012. Use of MISR/Terra data to study intra- and inter-annual EVI variations in the dry season of tropical forest. *Remote Sensing of Environment* **127**: 260-270.

Moya R, Tomazello Filho M. 2009. Wood density variation and tree ring demarcation in Gmelina arborea trees using x-ray densitometry. *Cerne*, **15(1)**, 92-100.

Muller-Landau HC. 2004. Interspecific and inter-site variation in wood specific gravity of tropical trees. *Biotropica* **36(1):** 20-32.

Nath C, Munoz F, Pélissier R, Burslem D, Muthusankar G. 2016. Growth rings in tropical trees: role of functional traits, environment, and phylogeny. *Trees* **30**: 2153-2175.

Nepveu G. **1976**. Croissance et qualité du bois de framiré. Evolution de la largeur de cerne et des composantes densitométriques en fonction de l'âge. *Bois et Forets des Tropiques* **165**: 39–58.

Nicolini E, Beauchêne J, de la Vallée BL, Ruelle J, Mangenet T & Heuret P. 2012. Dating branch growth units in a tropical tree using morphological and anatomical markers: the case of Parkia velutina Benoist (Mimosoïdeae). *Annals of forest science* **69(5):** 543-555.

Nock CA, Geihofer D, Grabner M, Baker PJ, Bunyavejchewin S, Hietz P. 2009. Wood density and its radial variation in six canopy tree species differing in shade-tolerance in western Thailand. *Annals of botany* **104:** 297–306.

Norby RJ, Zak DR. 2011. Ecological lessons from free-air CO2 enrichment (FACE) experiments. *Annual review of ecology, evolution, and systematics* **42:** 181-203.

Novak K, Sánchez MAS, Čufar K, Raventós J, de Luis M. 2013. Age, climate and intra-annual density fluctuations in Pinus halepensis in Spain. *Iawa Journal* **34(4):** 459-474.

O'Brien J, Oberbauer S, Clark D, Clark D. 2008. Phenology and Stem Diameter Increment Seasonality in a Costa Rican Wet Tropical Forest. *Biotropica* **40**: 151-159.

Pagotto MA, DeSoto L, Carvalho A, Nabais C, Tomazello Filho M, Ribeiro A, Lisi CS. 2017. Evaluation of X-ray densitometry to identify tree-ring boundaries of two deciduous species from semi-arid forests in Brazil. *Dendrochronologia* **42**: 94-103.

Paluš M, Novotná D, Tichavský P. 2005. Shifts of seasons at the European mid-latitudes: Natural fluctuations correlated with the North Atlantic Oscillation. *Geophysical research letters* **32(12)**.

Pan Y, Birdsey R, Fang J, Houghton R, Kauppi P, Kurz W, Phillips O, Shvidenko A, Lewis S, Canadell J *et al.* 2011. A Large and Persistent Carbon Sink in the World's Forests. *Science* **333**: 988-993.

Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, Blondel M, Prettenhofer P, Weiss R, Dubourg V, *et al.* 2012. Scikit-learn: Machine Learning in Python. *Journal of Machine Learning Research* **12**: 2825–2830.

Peel M, Finlayson B, McMahon T. 2007. Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences Discussions* **4**: 439-473.

Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte MS, Cornwell WK, Craine JM, Gurvich DE, Urcelay C, Veneklaas EJ, Reich PB, Poorter L, …, Urcelay C. 2013.

New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany* **61(3):** 167-234.

Peters RL, Groenendijk P, Vlam M, Zuidema PA. 2015. Detecting long-term growth trends using tree rings: a critical evaluation of methods. *Global change biology* **21(5):** 2040-2054.

Philippon N, de Lapparent B, Gond V, Sèze G, Martiny N, Camberlin P, Cornu G, Morel B, Moron V, Bigot S, Brou T, Dubreuil V. 2016. Analysis of the diurnal cycles for a better understanding of the mean annual cycle of forests greenness in Central Africa. *Agricultural and Forest Meteorology* **223**: 81-94.

Phillips OL, Aragão LE, Lewis SL, Fisher JB, Lloyd J, López-González G, Malhi Y, Monteagudo A, Peacock J, Quesada CA, van der Heijden G, ..., Torres-Lezama A. 2009. Drought sensitivity of the Amazon rainforest. *Science* **323(5919):** 1344-1347.

Phillips O, van der Heijden G, Lewis S, López-González G, Aragão L, Lloyd J, Malhi Y, Monteagudo A, Almeida S, Dávila E, Amaral I, …, Vilanova E. 2010. Drought-mortality relationships for tropical forests. *New Phytologist* **187**: 631-646.

Plourde BT, Boukili VK, Chazdon RL. **2015**. Radial changes in wood specific gravity of tropical trees: inter- and intraspecific variation during secondary succession. *Functional Ecology* **29**: 111–120.

Polge H. **1966**. Établissement des courbes de variation de la densité du bois par exploration densitométrique de radiographies d'échantillons prélevés à la tarière sur des arbres vivants. Applications dans les domaines Technologique et Physiologique. *Annales des sciences forestières* **23**: 215.

Poorter L, McDonald I, Alarcón A, Fichtler E, Licona JC, Peña-Claros M, Sterck F, Villegas Z, Sass-Klaassen U. **2010**. The importance of wood traits and hydraulic conductance for the performance and life history strategies of 42 rainforest tree species. *New Phytologist* **185**: 481–492.

Poussart PF, Evans MN & Schrag DP. 2004. Resolving seasonality in tropical trees: multi-decade, high-resolution oxygen and carbon isotope records from Indonesia and Thailand. *Earth and Planetary Science Letters* **218(3):** 301-316.

Poussart PM, Myneni SCB, Lanzirotti A. 2006. Tropical dendrochemistry: A novel approach to estimate age and growth from ringless trees. *Geophysical Research Letters* **33:** 1-5.

Prather MJ, Holmes CD, Hsu J. 2012. Reactive greenhouse gas scenarios: Systematic exploration of uncertainties and the role of atmospheric chemistry. *Geophysical Research Letters* **3(9)**.

Pucha-Cofrep D, Peters T, Bräuning A. 2015. Wet season precipitation during the past century reconstructed from tree-rings of a tropical dry forest in Southern Ecuador. *Global and Planetary Change* **133**: 65-78.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rana R, Langenfeld-Heyser R, Finkeldey R, Polle A. 2009. Functional anatomy of five endangered tropical timber wood species of the family Dipterocarpaceae. *Trees—Structure and Function* **23:** 521–529.

Reichstein M, Bahn M, Ciais P, Frank D, Mahecha MD, Seneviratne SI, Zscheischler J, Beer C, Buchmann N, Frank DC, Papale D, Rammig A, Smith P, Thonicke K, van der Velde M, Vicca S, Walz A, Wattenbach M. 2013. Climate extremes and the carbon cycle. *Nature* **500:** 287-295.

Reid WV, Mooney HA, Cropper A, Capistrano D, Carpenter SR, Chopra K. 2005. Millennium Ecosystem Assessment Synthesis Report. United Nations Environment Programme.

Ricker N. 1953. Wavelet contraction, wavelet expansion, and the control of seismic resolution. *Geophysics **18*(4)**: 769-792.

Rigozo N, Nordeman D, Echer E, Vieira L, Echer M, Prestes A. 2005. Tree-ring width wavelet and spectral analysis of solar variability and climatic effects on a Chilean cypress during the last two and a half millennia. *Climate of the Past Discussions* **1(1)**: 121-135.

Romero C, Bolker BM. 2008. Effects of stem anatomical and structural traits on responses to stem damage: an experimental study in the Bolivian Amazon. *Canadian Journal of Forest Research* **38:** 611–618.

Roque RM, Tomazelo-Filho M. **2007**. Relationships between anatomical features and intra-ring wood density profiles in Gmelina arborea applying X-ray densitometry. *Cerne* **13**: 384–392.

Rozendaal D, Zuidema P. 2011. Dendroecology in the tropics: a review. *Trees* **25**: 3-16.

Richardson AD, Keenan TF, Migliavacca M, Ryu Y, Sonnentag O, Toomey M. 2013. Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. *Agricultural and Forest Meteorology* **169:** 156-173.

Ruiz-Benito P, Ratcliffe S, Zavala MA, Martínez-Vilalta J, Vilà-Cabrera A, Lloret F, Madrigal-Gonzalez J, Wirth C, Greenwood S, Kändler G, Lehtonen, A, Kattge J, Dahlgren J, Jump AS. 2017. Climate-and successional-related changes in functional composition of European forests are strongly driven by tree mortality. *Global change biology* **23(10):** 4162-4176.

Sabine CL, Heimann M, Artaxo P, Bakker DCE, Chen CTA, …, Valentini R. 2004. Current Status and Past Trends of the Flobal Carbon Cycle. In The Global Carbon Cycle: Integrating Humans, Climate, and the Natural World, C. B. Field, M. R. Raupach, Eds. (Island Press, Washington, DC, 2004) pp. 17–44.

Sánchez-Carrillo S, Morea R, Serrano-Grijalva L, Meco A, Sánchez-Andrés R. 2015. A free air CO 2 enrichment (FACE) facility in a wetland to study the effects of elevated atmospheric carbon dioxide: system description and performance. *Wetlands* **35(1)**: 193-205.

Schaefli B, Zehe E. 2009. Hydrological model performance and parameter estimation in the wavelet-domain. *Hydrology and Earth System Sciences* **13(10)**: 1921.

Scheffer M, Carpenter S, Foley J, Folke C, Walker B. 2001. Catastrophic shifts in ecosystems. *Nature* **413**: 591-596.

Schierbeek A. 1959. Measuring the invisible world: the life and works of Antoni van Leeuwenhoek (No. 37). Abelard-Schuman Limited, London.

Schneider CA, Rasband WS, Eliceiri KW. **2012**. NIH Image to ImageJ : 25 years of image analysis. *Nature Methods* **9**: 671–675.

Schöngart J, Piedade MTF, Ludwigshausen S, Horna V, Worbes M. 2002. Phenology and stem-growth periodicity of tree species in Amazonian floodplain forests. *Journal of Tropical Ecology* **18:** 581– 597.

Schöngart J, Junk WJ, Piedade MTF, Ayres JM, Hüttermann A, Worbes M. 2004. Teleconnection between tree growth in the Amazonian floodplains and the El Niño-Southern oscillation effect. *Global Change Biology* **10:** 683–692.

Schöngart J, Orthmann B, Hennenberg KJ, Porembski S, Worbes M. **2006**. Climate-growth relationships of tropical tree species in West Africa and their potential for climate reconstruction. *Global Change Biology* **12**: 1139–1150.

Schöngart J, Wittmann F. 2010. Biomass and net primary production of central Amazonian floodplain forests. In Amazonian Floodplain Forests (pp. 347-388). Springer, Dordrecht.

Schweingruber FH. 1996. Tree rings and environment: dendroecology. Paul Haupt AG Bern. 609pp.

Schweingruber FH, Börner A, Schulze ED. 2006. Atlas of woody plant stems: evolution, structure, and environmental modifications. Springer, Heidelberg 229 pp.

Sexton J, Noojipady P, Song X, Feng M, Song D, Kim D, Anand A, Huang C, Channan S, Pimm S *et al.* 2016. Conservation policy and the measurement of forests. *Nature Climate Change* **6(2)**: 192-196.

Singh K, Kushwaha C. 2016. Deciduousness in tropical trees and its potential as indicator of climate change: A review. *Ecological indicators* **69:** 699-706.

Singh RK, Svystun T, AlDahmash B, Jönsson AM, Bhalerao RP. **2017**. Photoperiod- and temperature-mediated control of phenology in trees - a molecular perspective. *New Phytologist* **213**: 511–524.

Sitch S, Brovkin V, Bloh W, van Vuuren D, Eickhout B, Ganopolski A. 2005. Impacts of future land cover changes on atmospheric CO2 and climate. *Global Biogeochemical Cycles* **19(2)**.

Sonsin JO, Gasson PE, Barros CF, Marcati CR. 2012. A comparison of the wood anatomy of 11 species from two cerrado habitats (cerrado ss and adjacent gallery forest). *Botanical Journal of the Linnean Society* **170(2):** 257-276.

Sorce C, Giovannelli A, Sebastiani L, Anfodillo T. **2013**. Hormonal signals involved in the regulation of cambial activity, xylogenesis and vessel patterning in trees. *Plant Cell Reports* **32**: 885–898.

Sperry JS, Hacke UG, Pittermann J. 2006. Size and function in conifer tracheids and angiosperm vessels. *American Journal of Botany* **93:** 1490– 1500.

Spracklen DV, Arnold SR, Taylor CM. 2012. Observations of increased tropical rainfall preceded by air passage over forests. *Nature* **489:** 282–285.

Steffenrem A, Kvaalen H, Dalen KS, Høibø OA. **2014**. A high-throughput X-ray-based method for measurements of relative wood density from unprepared increment cores from Picea abies. *Scandinavian Journal of Forest Research* **29**: 506–514.

Studhalter RA, Glock WS, Agerter SR. 1963. Tree Growth, some historical chapters in the study of diameter growth. *Botanical Review* **29(3):** 245--343.

Subedi N, Sharma M. 2013. Climate-diameter growth relationships of black spruce and jack pine trees in boreal Ontario, Canada. *Global Change Biology* **19:** 505–516.

Swenson NG, Enquist BJ. 2007. Ecological and evolutionary determinants of a key plant functional trait: Wood density and its community-wide variation across latitude and elevation. *American Journal of Botany* **94(3):** 451-459.

Tarelkin Y, Delvaux C, De Ridder M, El Berkani T, De Cannière C, Beeckman H. 2016. Growth-ring distinctness based on IAWA's definition: variability and consequences for trait analysis. *IAWA Journal* **37**: 275-294.

Tarhule A, Hughes MK. 2002. Tree-ring research in semi-arid West Africa: need and potential. *Tree-Ring Research* **58(1/2):** 31-46.

Taylor PG, Cleveland CC, Wieder WR, Sullivan BW, Doughty CE, Dobrowski SZ, Townsend AR. 2017. Temperature and rainfall interact to control carbon cycling in tropical forests. *Ecology letters* **20(6):** 779-788.

Tepper HB, Hollis CA. 1967. Mitotic reactivation of the terminal bud and cambium of white ash. *Science* **156(3782):** 1635--1636.

Tranvik LJ, Downing JA, Cotner JB, Loiselle SA, Striegl RG, Ballatore TJ, Dillon P, Finlay K, Fortino K, Knoll LB, Kortelainen PL, Kutser T, …, Weyhenmeyer GA. 2009. Lakes and reservoirs as regulators of carbon cycling and climate. *Limnology and Oceanography* **54(6part2):** 2298-2314.

Turner BL, Lambin EF, Reenberg A. 2007. The emergence of land change science for global environmental change and sustainability. *Proceedings of the National Academy of Sciences* **104(52):** 20666-20671.

Valentini R, Arneth A, Bombelli A, Castaldi S, Cazzolla Gatti R, Chevallier F, Ciais P, Grieco E, Hartmann J, Henry M, Houghton RA, Jung M, Kutsch WL, Malhi Y, …, Scholes RJ. 2014. A full greenhouse gases budget of Africa: synthesis, uncertainties, and vulnerabilities. *Biogeosciences* **11:** 381-407.

Vancutsem C, Pekel J, Kibambe L, Blaes X, de Waseige C, Defourny F. 2006. République démocratique du Congo - occupation du sol. Carte Géographique. Presses Universitaire de Louvain. Bruxelles, Belgique.

Van den Bulcke J, Boone MN, Van Acker J, Stevens M, Van Hoorebeke L. **2009**. Van den Bulcke J, Boone MN, Van Acker J, Stevens M, Van Hoorebeke L. 2009. X-ray tomography as a tool for detailed anatomical analysis. Ann. For. Sci. 66: 508.X-ray tomography as a tool for detailed anatomical analysis. *Ann. For. Sci.* **66**: 508.

Van den Bulcke J, Wernersson ELG, Dierick M, Van Loo D, Masschaele B, Brabant L, Boone MN, Van Hoorebeke L, Haneca K, Brun A, *et al.* 2014. 3D tree-ring analysis using helical X-ray tomography. *Dendrochronologia* **32**: 39–46.

Van Der Sleen P, Groenendijk P, Vlam M, Anten NP, Boom A, Bongers F, Pons TL, Terburg G, Zuidema PA. 2015. No growth stimulation of tropical trees by 150 years of CO2 fertilization but water-use efficiency increased. *Nature geoscience* **8(1):** 24-28.

Van Der Walt S, Schönberger JL, Nunez-Iglesias J, Boulogne F, Warner JD, Yager N, Gouillart E, Yu T. **2014**. scikit-image: image processing in Python. *PeerJ* **2**: e453.

van der Werf G, Morton D, DeFries R, Olivier J, Kasibhatla P, Jackson R, Collatz G, Randerson J. 2009. CO2 emissions from forest loss. *Nature Geoscience* **2**: 737-738.

Van Ranst E, Baert G, Ngongo M & Mafuka P. 2010. Carte pédologique de Yangambi, planchette 2: Yangambi, échelle 1:50.000. Published by UGent, Hogent, Ghent, Belgium

Vancutsem C, Pekel J, Kibambe L, Blaes X, de Waseige C, Defourny F. 2006. *République démocratique du Congo - occupation du sol.* Carte Géographique. Presses Universitaire de Louvain. Bruxelles, Belgique.

Vargo J, Habeeb D, Stone Jr. B. 2013. The importance of land cover change across urban-rural typologies for climate modelling. *Journal of Environmental Management* **114:** 243-252.

Venegas-González A, von Arx G, Chagas MP, Tomazello Filho M. 2015. Plasticity in xylem anatomical traits of two tropical species in response to intra-seasonal climate variability. *Trees* **29(2):** 423-435.

Viennois G, Barbier N, Fabre I, Couteron P. 2013. Multiresolution quantification of deciduousness in West-Central African forests. *Biogeosciences* **10**: 6957-6967.

Villar-Salvador P, Castro-Díez P, Pérez-Rontomé C, Montserrat-Martí G. 1997. Stem xylem features in three Quercus (Fagaceae) species along a climatic gradient in NE Spain. *Trees* **12**: 90-96.

Violle C, Navas ML, Vile D, Kazakou E, Fortunel C, Hummel I, Garnier E. 2007. Let the concept of trait be functional! *Oikos* **116(5):** 882-892.

Vlam M, Baker P, Bunyavejchewin S, Zuidema P. 2014. Temperature and rainfall strongly drive temporal growth variation in Asian tropical forest trees. *Oecologia* **174**: 1449-1461.

Vlassenbroeck J, Dierick M, Masschaele B, Cnudde V, Van Hoorebeke L, Jacobs P. 2007. Software tools for quantification of X-ray microtomography at the UGCT. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment* **580**: 442-445.

von Arx G, Carrer M. 2014. ROXAS–A new tool to build centuries-long tracheid-lumen chronologies in conifers. *Dendrochronologia* ***32*****(3)**: 290-293.

von Schnakenburg P, Bräuning A, Helle G. 2008. Detecting annual growth rhythms from high-frequency densitometry and carbon isotopes in tropical mountain rain forest trees in southern Ecuador. *TRACE - Tree Rings in Archaeology, Climatology and Ecology* **6**: 96-99.

Way DA, Oren R. 2010. Differential responses to changes in growth temperature between trees from different functional groups and biomes: a review and synthesis of data. *Tree Physiology* **30:** 669–688.

Wheeler E, Baas P, Rodgers S. 2007. Variations In Dicot Wood Anatomy: A Global Analysis Based on the Insidewood Database. *IAWA Journal* **28**: 229-258.

Wheeler EA. 2011. Inside wood–a web resource for hardwood anatomy. *Iawa Journal* **32(2):** 199-211.

Wheeler EA, Baas P. **1993**. The potentials and limitations of dicotyledonous wood anatomy for climatic reconstructions. *Paleobiology* **19**: 487–498.

Whitmore TC. 1998. An introduction to tropical rain forests. Oxford University Press, Oxford, United Kingdom.

Wiemann MC, Williamson GB. 2007. Extreme radial changes in wood specific gravity in some tropical pioneers. *Wood and Fiber science* **20(3):** 344-349.

Wiemann M, Wheeler E, Manchester S, Portier K. 1998. Dicotyledonous wood anatomical characters as predictors of climate. *Palaeogeography, Palaeoclimatology, Palaeoecology* **139**: 83-100.

Wils TH, Robertson I, Eshetu Z, Sass-Klaassen UG, Koprowski M. 2009. Periodicity of growth rings in Juniperus procera from Ethiopia inferred from crossdating and radiocarbon dating. *Dendrochronologia* **27(1):** 45-58.

Wils TH, Sass-Klaassen UG, Eshetu Z, Bräuning A, Gebrekirstos A, Couralet C, Robertson I, Touchan R, Koprowski M, Conway D. 2011. Dendrochronology in the dry tropics: the Ethiopian case. *Trees* **25:** 345–354.

Wimmer R. 2002. Wood anatomical features in tree-rings as indicators of environmental change. *Dendrochronologia* **20**: 21-36.

Woodcock D, Shier A. 2002. Wood specific gravity and its radial variations: the many ways to make a tree. *Trees* **16(6):** 437-443.

Worbes M. 1985. Structural and other adaptations to longterm flooding by trees in Central Amazonia. *Amazoniana* **9:** 459–484.

Worbes M. 1995. How to Measure Growth Dynamics in Tropical Trees? A Review. *IAWA Journal* **16**: 337-351.

Worbes M. **1995**. How To Measure Growth Dynamics in Tropical Trees. *IAWA Journal* **16**: 337–351.

Worbes M. 1999. Annual growth rings, rainfall-dependent growth and long-term growth patterns of tropical trees from the Caparo Forest Reserve in Venezuela. *Journal of Ecology* **87(3):** 391-403.

Worbes M, Junk WJ. 1999. How old are tropical trees? The persistence of a myth. *IAWA Journal* **20(3):** 255–260.

Worbes M. **2002**. One hundred years of tree-ring research in the tropics – a brief history and an outlook to future challenges. *Dendrochronologia* **20**: 217–231.

Worbes M, Fichtler E. 2010. Wood anatomy and tree-ring structure and their importance for tropical dendrochronology. In: Junk, WJ, Piedade, MTF, Wittmann, F, Schöngart, J, Parolin, P (Eds.), Amazonian Floodplain Forests: Ecophysiology, Biodiversity and Sustainable Management. Springer, Berlin/ Heidelberg/New York, pp. 329--346.

Worbes M, Blanchart S, Fichtler E. **2013**. Relations between water balance, wood traits and phenological behavior of tree species from a tropical dry forest in Costa Rica--a multifactorial study. *Tree Physiology* **33**: 527–36.

Wu K, Otoo E, Shoshani A. 2005. Optimizing connected component labeling algorithms. In *Medical Imaging 2005: Image Processing* **5747:** 1965-1977. International Society for Optics and Photonics.

Wu J, Albert LP, Lopes AP, Restrepo-Coupe N, Hayek M, Wiedemann KT, Guan K, Stark SC, Christoffersen B, Prohaska N, Tavares JV, …, Saleska SR. 2016. Leaf development and demography explain photosynthetic seasonality in Amazon evergreen forests. *Science* **351(6276):** 972-976.

WRB (World reference base for soil resources). 2014. *International soil classification system for naming soils and creating legends for soil maps. World Soil Reports*, **(106)**. FAO. Rome

Wright SJ, Yavitt JB, Wurzburger N, Turner BL, Tanner EV, Sayer EJ, Santiago LS, Kaspari M, Hedin LO, Harms KE, Garcia MN, Corre MD. 2011. Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. *Ecology* **92(8):** 1616-1625.

Zanne A, Westoby M, Falster D, Ackerly D, Loarie S, Arnold S, Coomes D. 2010. Angiosperm wood structure: Global patterns in vessel anatomy and their relation to wood density and potential conductivity. *American Journal of Botany* **97**: 207-215.

Zhang SB, Slik JWF, Zhang JL, Cao KF. 2011. Spatial patterns of wood traits in China are controlled by phylogeny and the environment. *Global Ecology and Biogeography* **20:** 241–250.

Ziemińska K, Butler DW, Gleason SM, Wright IJ, Westoby M. **2013**. Fibre wall and lumen fractions drive wood density variation across 24 Australian angiosperms. *AoB PLANTS* **5**: plt046-plt046.

Ziemińska K, Westoby M, Wright IJ. **2015**. Broad anatomical variation within a narrow wood density range - A study of twig wood across 69 Australian angiosperms. *PLoS ONE* **10**: 1–25.

Zuidema P, Baker P, Groenendijk P, Schippers P, van der Sleen P, Vlam M, Sterck F. 2013. Tropical forests and global change: filling knowledge gaps. *Trends in Plant Science* **18**: 413-419.

Supplementary material

Annex 1 : List of species and the description of all their ring boundaries of the RMCA collection studied in Paper 1

Annex 1

Species name	Tw-number	Site	# tree ring	AC_FW	AC_FD	GC_FD	GC_VD	VDV	MP	PDV	DR	D	I	A
Agelaea pentagyna	32401	Luki	1	0	1	0	1	0	0	0	0	1	0	0
Agelaea pentagyna		Luki	2	0	1	0	0	0	0	0	0	1	0	0
Agelaea pentagyna	32433	Luki	1	0	1	0	0	0	0	0	0	1	0	0
Agelaea pentagyna	32836	Ygbi	0									0	0	1
Agelaea pentagyna	33484	Ygbi	1	0	1	0	0	0	0	0	0	1	0	0
Agelaea pentagyna			2	0	1	0	0	0	0	0	0	1	0	0
Agelaea pentagyna			3	0	1	0	1	0	0	0	0	1	0	0
Agelaea pentagyna	35031	Ygbi	1	0	1	0	1	0	0	0	0	1	0	0
Agelaea pentagyna	41291	Luki	0									0	0	1
Albizia ferruginea	1172	Ygbi	0									0	0	1
Albizia ferruginea	1450	Luki	0									0	0	1
Albizia ferruginea	3620	Ygbi	0									0	0	1
Albizia gummifera	1111	Ygbi	1	0	0	1	0	0	0	0	0	0	1	0
Albizia gummifera	2164	Ygbi	1	0	0	1	0	0	0	0	0	0	1	0
Albizia gummifera	1141	Ygbi	1	0	0	0	0	0	0	0	0	0	1	0
Alstonia boonei	1470	Luki	1	1	1	0	0	0	1	1	0	1	0	0
			2	1	0	0	0	0	1	1	0	1	0	0
			3	1	0	0	0	0	1	1	0	1	0	0
Alstonia boonei	5203	Ygbi	1	0	1	0	0	0	1	1	0	1	0	0
			2	0	1	0	0	0	1	1	0	1	0	0
			3	0	1	0	0	0	1	1	0	1	0	0
Alstonia boonei	5208	Ygbi	1	0	1	0	0	0	1	1	0	1	0	0
			2	0	1	0	0	0	1	0	0	1	0	0
			3	0	1	0	0	0	1	0	0	1	0	0
			4	0	1	0	0	0	1	0	0	1	0	0
Alstonia boonei	24320	Ygbi	1	0	0	0	0	0	1	1	0	0	1	0
Alstonia congensis	1147	Ygbi	1	0	1	0	0	0	1	0	0	1	0	0

Alstonia congensis	1682	Luki	0									0	0	1
Annea laxiflora	32307	Luki	0									0	0	1
Annea laxiflora	32418	Luki	1	0	1	0	0	0	0	0	0	1	0	0
			2	0	1	0	0	0	0	0	0	1	0	0
			3	0	1	0	0	0	0	0	0	1	0	0
			4	0	1	0	0	0	0	0	0	1	0	0
Annea laxiflora	32424	Luki	1	0	1	0	0	0	0	0	0	1	0	0
			2	0	1	0	0	0	0	0	0	1	0	0
			3	0	1	0	0	0	0	0	0	1	0	0
			4	0	1	0	0	0	0	0	0	1	0	0
			5	0	1	0	0	0	0	0	0	1	0	0
Anonidium mannii	72	Luki	1	1	0	0	0	0	0	0	1	1	0	0
			2	1	0	0	0	0	0	0	1	1	0	0
Anonidium mannii	1191	Ygbi	1	0	1	0	0	0	0	0	1	1	0	0
			2	0	1	0	0	0	0	0	1	1	0	0
			3	0	1	0	0	0	0	0	1	1	0	0
Anonidium mannii	26314	Ygbi	1	0	0	0	0	0	0	0	1	0	0	0
Antiaris toxicaria	1153	Ygbi	0									0	0	1
Antiaris toxicaria	1428	Luki	0									0	0	1
Antiaris toxicaria	5152	Luki	0									0	0	1
Baikiaea insignis	2432	Ygbi	0									0	0	1
Baikiaea insignis	24394	Ygbi	1	0	1	0	0	0	1	0	0	1	0	0
			1	0	1	0	0	0	1	0	0	1	0	0
			1	0	1	0	0	0	1	0	0	1	0	0
Baikiaea insignis	32245	Luki	0									0	0	1
Baikiaea insignis	32535	Luki	0									0	0	1
Baissea axillaris	32527	Luki	0									0	0	1
Baissea axillaris	32671	Luki	0									0	0	1
Baissea axillaris	33528	Ygbi	0									0	0	1

Berlinia bracteosa	1181	Luki	0									0	0	1
Berlinia bracteosa	4837	Luki	1	0	0	0	0	0	1	0	0	0	1	0
			2	0	0	0	0	0	1	0	0	0	1	0
Blighia welwitschii	179	Luki	1	0	1	0	0	0	0	0	0	0	1	0
			2	0	1	0	0	0	0	0	0	0	1	0
Blighia welwitschii	1116	Ygbi	1	0	1	0	0	0	0	0	0	1	0	0
			2	0	1	0	0	0	0	0	0	1	0	0
Blighia welwitschii	1424	Luki	1	0	1	0	0	0	0	0	0	1	0	0
Canarium schweinfurthii	128	Luki	0									0	0	1
Canarium schweinfurthii	1164	Ygbi	0									0	0	1
Canarium schweinfurthii	1452	Luki	0									0	0	1
Carpolobia alba	32934	Ygbi	1	0	0	0	0	0	1	1	0	0	1	0
Carpolobia alba	32996	Ygbi	1	0	0	0	0	0	1	1	0	0	1	0
Carpolobia alba	33041	Ygbi	0									0	0	1
Celtis mildbraedii	5159	Luki	1	0	0	1	0	0	0	0	1	0	1	0
Celtis mildbraedii	5242	Ygbi	0									0	0	1
Chrysophyllum africanum	1132	Ygbi	0									0	0	1
Chrysophyllum africanum	1135	Ygbi	0									0	0	1
Chrysophyllum africanum	33809	Ygbi	1	0	1	0	0	1	1	0	0	0	1	0
Chytranthus macrobotrys	32573	Luki	0									0	0	1
Chytranthus macrobotrys	35555	Ygbi	1	0	1	0	0	0	1	0	0	1	0	0
Chytranthus macrobotrys	35718	Ygbi	1	0	1	0	0	0	1	0	0	1	0	0
			2	0	1	0	0	0	1	0	0	1	0	0
Chytranthus macrobotrys	68426	Ygbi	1	0	1	0	0	0	1	0	0	1	0	0
			2	0	1	0	0	0	1	0	0	1	0	0
			3	0	1	0	0	0	1	0	0	1	0	0
			4	0	1	0	0	0	1	0	0	1	0	0
			5	0	1	0	0	0	1	0	0	1	0	0
Cnestis ferruginea	68407	Ygbi	1	0	1	0	0	0	0	0	0	1	0	0

			2	0	1	0	0	0	0	0	0	1	0	0
			3	0	1	0	1	0	0	0	0	1	0	0
			4	0	1	0	1	0	0	0	0	1	0	0
			5	0	1	0	0	0	0	0	0	1	0	0
Cola bruneelii	61083	Luki	1	0	0	0	0	0	1	0	1	0	1	0
Connarus griffonianus	32327	Luki	0									0	0	1
Connarus griffonianus	32378	Luki	0									0	0	1
Connarus griffonianus	33598	Ygbi	0									0	0	1
Copaifera mildbraedii	33153	Ygbi	1	0	0	1	0	0	0	0	0	0	1	0
			2	1	0	0	0	0	0	0	1	0	1	0
Copaifera mildbraedii	33538	Ygbi	1	1	0	0	0	0	0	0	1	0	1	0
			2	1	0	0	0	0	0	0	1	0	1	0
Copaifera mildbraedii	34715	Ygbi	1	1	0	0	0	0	0	0	1	0	1	0
Copaifera mildbraedii	34814	Ygbi	1	1	0	0	0	0	0	0	1	0	1	0
Copaifera mildbraedii	68422	Ygbi	0									0	0	1
Cynometra lujae	1440	Luki	0									0	0	1
Cynometra lujae	4830	Luki	0									0	0	1
Cynometra lujae	32258	Luki	1	0	0	0	0	0	1	0	1	0	1	0
Cynometra lujae	32259	Luki	1	1	1	0	0	0	1	0	1	1	0	0
Cynometra lujae	32273	Luki	1	0	1	0	0	0	0	0	1	1	0	0
Cynometra lujae	32323	Luki	1	0	1	0	0	0	0	0	1	1	0	0
			2	0	1	0	0	0	0	0	1	1	0	0
Cynometra lujae	32566	Luki	1	0	1	0	0	0	1	0	1	1	0	0
Dalhousiea africana	32562	Luki	0									0	0	1
Dalhousiea africana	32893	Ygbi	0									0	0	1
Dalhousiea africana	41266	Luki	0									0	0	1
Dewevrea bilabiata	32604	Luki	0									0	0	1
Dewevrea bilabiata	32650	Luki	0									0	0	1
Dewevrea bilabiata	33573	Ygbi	0									0	0	1

Dialium corbisieri	5206	Ygbi	1	0	0	0	0	0	1	0	1	0	1	0
Dialium corbisieri	5245	Ygbi	1	0	0	0	0	0	1	0	1	0	1	0
			2	0	0	0	0	0	1	0	1	0	1	0
			3	0	0	0	0	0	1	0	1	0	1	0
Dialium excelsum	1107	Ygbi	1	0	1	0	0	0	1	0	1	0	1	0
Dialium excelsum	2429	Ygbi	1	0	0	0	0	0	1	0	1	0	1	0
Dialium excelsum	3612	Ygbi	1	0	0	0	0	0	1	0	1	0	1	0
			2	0	0	0	0	0	1	0	1	0	1	0
Dialium tessmannii	32248	Luki	1	0	0	0	0	0	1	0	0	0	1	0
			2	0	0	0	0	0	1	0	0	0	1	0
Dialium tessmannii	32324	Luki	1	0	0	0	0	0	1	0	0	0	1	0
			2	0	0	0	0	0	1	1	0	0	1	0
Dichapetalum acuminatum	32776	Ygbi	0									0	0	1
Dichapetalum acuminatum	32778	Ygbi	0									0	0	1
Dichapetalum acuminatum	33733	Ygbi	0									0	0	1
Dichapetalum acuminatum	34843	Ygbi	0									0	0	1
Dichapetalum acuminatum	35503	Ygbi	1	0	1	0	0	1	0	0	0	1	0	0
Dichapetalum congoense	33491	Ygbi	0									0	0	1
Dichapetalum congoense	35909	Ygbi	1	1	1	0	0	0	0	0	0	0	1	0
Dichapetalum congoense	38273	Ygbi	0									0	0	0
Dichapetalum congoense	38388	Ygbi	1	0	0	0	0	1	0	0	0	0	1	0
Dichapetalum heudelotii	32598	Luki	1	0	0	0	0	1	0	0	0	0	1	0
Dichapetalum heudelotii	32781	Ygbi	1	0	0	0	0	1	0	0	0	0	1	0
Dichapetalum heudelotii	32787	Ygbi	1	1	0	0	0	0	0	0	0	1	0	0
Dichapetalum heudelotii	33272	Ygbi	0									0	0	0
Dichapetalum heudelotii	33618	Ygbi	0									0	0	1
Dichapetalum madagascariense	32237	Luki	1	0	0	0	0	1	0	0	0	0	1	0
Dichapetalum madagascariense	32344	Luki	0									0	0	1
Dichapetalum madagascariense	32541	Luki	0									0	0	1

Dichapetalum madagascariense	32783	Ygbi	1	0	0	0	0	1	0	0	0	0	1	0
Dichapetalum madagascariense	32785	Ygbi	0									0	0	1
Dichapetalum madagascariense	32790	Ygbi	1	0	0	1	0	1	0	0	0	0	1	0
			2	0	0	1	0	1	0	0	0	0	1	0
			3	0	0	1	0	1	0	0	0	0	1	0
			4	0	0	1	0	1	0	0	0	0	1	0
Dichapetalum madagascariense	32792	Ygbi	0									0	0	1
Dichapetalum madagascariense	32916	Ygbi	1	0	0	0	0	1	1	0	0	0	1	0
Dichapetalum madagascariense	32989	Ygbi	1	1	0	0	0	1	1	0	0	0	1	0
Dichapetalum madagascariense	33078	Ygbi	0									0	0	1
Dichapetalum madagascariense	33217	Ygbi	0									0	0	1
Dichapetalum madagascariense	33251	Ygbi	1	0	0	0	0	1	0	0	0	0	1	0
Dichapetalum mombuttense	32789	Ygbi	0									0	0	1
Dichapetalum mombuttense	32905	Ygbi	1	1	0	0	0	0	0	0	0	0	1	0
Dichapetalum mombuttense	33221	Ygbi	1	0	1	0	0	0	1	0	0	1	1	0
Dichapetalum mombuttense	35639	Ygbi	0									0	0	0
Dichapetalum mombuttense	35762	Ygbi	1	0	0	0	0	0	0	0	0	0	1	0
Diospyros pseudomespilus	32194	Luki	0									0	0	1
Diospyros pseudomespilus	32797	Luki	1	0	0	0	0	0	0	0	0	0	1	0
Diospyros pseudomespilus	35481	Ygbi	1	1	0	0	1	1	0	0	0	1	1	0
			2	1	0	0	1	1	0	0	0	1	1	0
Diospyros pseudomespilus	38338	Ygbi	0									0	0	1
Dracaena mannii	32388	Luki	0									0	0	1
Dracaena mannii	33683	Ygbi	0									0	0	1
Empogona crepiniana	32920	Ygbi	0									0	0	1
Empogona crepiniana	34890	Ygbi	0									0	0	1
Entandrophragma angolense	43	Luki	1	1	0	0	0	0	1	0	1	0	1	0
			2	1	0	0	0	0	1	0	1	0	1	0
Entandrophragma angolense	133	Luki	1	1	0	0	0	0	1	0	1	0	1	0

			2	1	0	0	0	0	1	0	1	0	1	0
Entandrophragma angolense	1118	Ygbi	1	0	0	0	0	0	1	0	1	0	1	0
			2	0	0	0	0	0	1	0	1	0	1	0
Entandrophragma angolense	1212	Luki	1	1	0	0	0	0	1	0	1	0	1	0
Entandrophragma angolense	1432	Luki	1	1	0	0	0	0	1	0	1	0	1	0
Entandrophragma utile	134	Luki	1	0	0	0	0	0	1	0	1	0	1	0
		Luki	2	0	0	0	0	0	1	0	1	0	1	0
Entandrophragma utile	1102	Ygbi	1	0	0	0	0	0	1	0	1	0	1	0
			2	0	0	0	0	0	1	0	1	0	1	0
Entandrophragma utile	1410	Luki	1	0	1	0	0	0	1	0	1	1	0	0
		Luki	2	0	1	0	0	0	1	0	1	1	0	0
		Luki	3	0	1	0	0	0	1	0	1	1	0	0
		Luki	4	0	1	0	0	0	1	0	1	1	0	0
		Luki	5	0	1	0	0	0	1	0	1	1	0	0
Erythrophleum suaveolens	96	Luki	0									0	0	0
Erythrophleum suaveolens	1101	Ygbi	0	0	0	0	0	0	1	0	0	0	1	0
Erythrophleum suaveolens	1187	Ygbi	0									0	0	0
Erythrophleum suaveolens	3619	Ygbi	1	0	0	0	0	0	1	1	0	0	1	0
Ganophyllum giganteum	5151	Luki	1									0	0	0
			2	0	1	0	0	0	1	0	0	1	0	0
			3	0	1	0	0	0	1	0	0	1	0	0
Ganophyllum giganteum	32217	Luki	0									0	0	1
Ganophyllum giganteum	32526	Luki	1	1	1	0	0	0	0	0	0	1	0	0
			2	1	1	0	0	0	0	0	0	1	0	0
			3	1	1	0	0	0	0	0	0	1	0	0
Hugonia platysepala	24325	Ygbi	0									0	0	1
Hugonia platysepala	32387	Luki	0									0	0	1
Hugonia platysepala	32517	Luki	1	0	1	0	0	0	1	0	0	1	0	0
Hylodendron gabunense	1275	Luki	1	0	1	0	0	0	0	0	0	1	0	0

Hylodendron gabunense	1364	Luki	1	0	0	0	0	0	0	0	0	1	0	0
			2	0	0	0	0	0	0	0	0	1	0	0
			3	0	0	0	0	0	0	0	0	1	0	0
			4	0	0	0	0	0	0	0	0	1	0	0
Hylodendron gabunense	1422	Luki	1	0	0	0	0	0	1	0	0	1	0	0
			2	0	0	0	0	0	1	0	0	1	0	0
			3	0	0	0	0	0	1	0	0	1	0	0
Hylodendron gabunense	4832	Luki	1	0	0	0	0	1	1	0	0	0	1	0
			2	0	0	0	0	1	0	0	0	0	1	0
			3	0	0	0	0	1	1	0	0	0	1	0
			4	0	0	0	0	1	1	0	0	0	1	0
			5	0	0	0	0	1	1	0	0	0	1	0
Hylodendron gabunense	32211	Luki	0									0	0	1
Irvingia grandifolia	1117	Ygbi	1	0	0	1	0	0	1	0	0	0	1	0
Irvingia grandifolia	1288	Luki	1	0	0	0	0	0	1	1	0	0	1	0
Irvingia grandifolia	1299	Luki	0									0	0	1
Irvingia grandifolia	1310	Luki	0									0	0	1
Irvingia grandifolia	1415	Luki	0									0	0	1
Isolona hexaloba	33242	Ygbi	1	0	1	0	0	0	1	0	1	1	0	0
			2	0	1	0	0	0	1	0	1	1	0	0
			3	0	1	0	0	0	1	0	1	1	0	0
			4	0	1	0	0	0	1	0	1	1	0	0
Isolona hexaloba	33298	Ygbi	1	0	0	0	0	1	0	0	0	0	1	0
			2	0	0	0	0	1	0	0	0	0	1	0
			3	0	1	0	0	1	0	0	0	1	0	0
Isolona hexaloba	33468	Ygbi	1	0	1	0	0	0	1	0	1	1	0	0
			2	0	1	0	0	0	1	0	1	1	0	0
Isolona hexaloba	68409	Ygbi	1	0	0	0	0	0	1	0	1	0	1	0
			2	0	0	0	0	0	1	0	1	0	1	0

			3	0	0	0	0	0	1	0	1	0	1	0
Julbernardia brieyi	111	Luki	1	0	0	0	0	0	1	0	1	0	1	0
			2	0	0	0	0	0	1	0	1	0	1	0
Julbernardia brieyi	1441	Luki	1	0	0	0	0	0	1	0	0	0	1	0
			2	0	0	0	0	0	1	0	0	0	1	0
			3	0	0	0	0	0	1	0	0	0	1	0
			4	0	1	0	0	0	0	0	0	1	0	0
Julbernardia brieyi	30657	Luki	0									0	0	0
Klainedoxa gabonensis	71	Luki	0									0	0	1
Klainedoxa gabonensis	1435	Luki	0									0	0	1
Klainedoxa gabonensis	33576	Ygbi	0									0	0	1
Klainedoxa gabonensis	35873	Ygbi	0									0	0	1
Klainedoxa gabonensis	68404	Ygbi	0									0	0	1
Lannea welwitschii	106	Luki	1	0	1	0	0	0	0	0	0	0	1	0
Lannea welwitschii	126	Luki	0									0	0	1
Lannea welwitschii	1464	Luki	1	0	1	0	0	0	0	0	1	1	0	0
			2	0	1	0	0	0	0	0	1	1	0	0
Lannea welwitschii	5158	Luki	1	0	1	0	0	0	0	0	0	0	1	0
Leplaea cedrata	85	Luki	0									0	0	1
Leplaea cedrata	1305	Luki	0									0	0	1
Leplaea cedrata	5246	Ygbi	0									0	0	1
Maesopsis eminii	1103	Ygbi	0									0	0	1
Maesopsis eminii	1170	Ygbi	0									0	0	1
Maesopsis eminii	1192	Luki	0									0	0	1
Mallotus oppositifolius	33687	Ygbi	0									0	0	1
Mallotus oppositifolius	35162	Ygbi	0									0	0	1
Malouetia bequaertiana	33191	Ygbi	1	0	1	0	0	0	0	0	0	1	0	0
Malouetia bequaertiana	33292	Ygbi	1	0	0	1	0	0	0	0	0	0	1	0
			2	0	0	1	0	0	0	0	0	0	1	0

			3	0	0	1	0	0	0	0	0	0	1	0
Malouetia bequaertiana	34946	Ygbi	1	0	0	1	0	0	0	0	0	0	1	0
Manotes expansa	32223	Luki	0									0	0	1
Manotes expansa	32363	Luki	1	0	1	0	0	0	0	0	0	1	0	0
			2	0	1	0	0	0	0	0	0	1	0	0
			3	0	1	0	0	0	1	0	0	1	0	0
Manotes expansa	32813	Ygbi	0									0	0	1
Maranthes glabra	70	Luki	0									0	0	1
Maranthes glabra	103	Luki	0									0	0	0
Maranthes glabra	5162	Luki	0									0	0	1
Maranthes glabra	32442	Luki	1	1	1	0	0	1	1	0	0	1	0	0
Maranthes glabra	32518	Luki	0									0	0	1
Maranthes glabra	32609	Luki	0									0	0	1
Margaritopsis oddonii	33206	Ygbi	0									0	0	1
Margaritopsis oddonii	33745	Ygbi	0									0	0	1
Margaritopsis oddonii	35773	Ygbi	0									0	0	1
Margaritopsis oddonii	38497	Ygbi	1	0	0	1	0	0	0	0	0	0	1	0
			2	0	0	1	0	0	0	0	0	0	1	0
			3	0	0	1	0	0	0	0	0	0	1	0
Microdesmis yafungana	32817	Ygbi	0									0	0	1
Microdesmis yafungana	33147	Ygbi	0									0	0	1
Milicia excelsa	1462	Luki	1	0	0	0	0	0	1	0	0	0	1	0
Milicia excelsa	1463	Luki	0									0	0	0
Monodora angolensis	80	Luki	1	1	0	0	0	0	0	0	1	1	0	0
Monodora angolensis	26316	Ygbi	1	1	0	0	0	0	0	0	1	1	0	0
			2	1	0	0	0	0	0	0	1	1	0	0
			3	1	1	0	0	0	0	0	1	1	0	0
Monodora myristica	87	Luki	1	1	0	0	0	0	1	0	1	1	0	0
			2	1	0	0	0	0	1	0	1	1	0	0

			3	1	0	0	0	0	1	0	1	0	1	0
Monodora myristica	1125	Ygbi	1	1	1	0	0	0	1	0	1	1	0	0
Monodora myristica	1279	Luki	1	1	0	0	0	0	1	0	1	1	0	0
			2	1	0	0	0	0	1	0	1	1	0	0
			3	1	0	0	0	0	1	0	1	1	0	0
			4	1	0	0	0	0	1	0	1	1	0	0
Nesogordonia kabingaensis	129	Luki	0									0	0	0
Nesogordonia kabingaensis	32544	Luki	0									0	0	0
Nesogordonia kabingaensis	32552	Luki	0									0	0	0
Nichallea soyauxii	32837	Ygbi	1	0	0	0	0	0	0	0	0	0	1	0
			2	1	0	0	0	0	0	0	0	0	1	0
			3	0	0	0	0	0	0	0	0	0	1	0
Nichallea soyauxii	32960	Ygbi	0									0	0	0
Nichallea soyauxii	33267	Ygbi	0									0	0	1
Nichallea soyauxii	34928	Ygbi	1	0	1	0	0	0	0	0	0	0	1	0
Nichallea soyauxii	38494	Ygbi	1	0	0	0	0	1	1	1	0	0	1	0
			2	0	0	0	0	1	1	1	0	0	1	0
Octolobus spectabilis	32252	Luki	0									0	0	0
Octolobus spectabilis	32468	Luki	0									0	0	0
Oncoba welwitschii	144	Luki	1	0	1	0	0	0	0	0	0	0	1	0
			2	1	0	0	0	0	0	0	1	0	1	0
Oncoba welwitschii	180	Luki	1	0	1	0	0	0	0	0	1	1	0	0
			2	0	1	0	0	0	0	0	1	1	0	0
Oncoba welwitschii	1300	Luki	1	0	1	0	0	0	0	0	0	0	1	0
			2	0	1	0	0	0	0	0	0	0	1	0
Oncoba welwitschii	24328	Ygbi	1	1	1	0	0	0	0	0	0	1	0	0
Ongokea gore	824	Luki	0									0	0	1
Ongokea gore	1145	Ygbi	0									0	0	1
Ongokea gore	1436	Luki	0									0	0	1

Oxyanthus speciosus	32515	Luki	1	0	0	0	1	1	0	0	0	0	1	0
			2	0	0	0	1	1	0	0	0	0	1	0
Oxyanthus speciosus	32546	Luki	1	0	0	0	0	1	0	0	0	0	1	0
			2	0	0	1	0	1	0	0	0	0	1	0
			3	0	0	1	0	1	0	0	0	0	1	0
			4	0	0	1	0	1	0	0	0	0	1	0
Oxyanthus speciosus	33414	Ygbi	0									0	0	1
Oxyanthus subpunctatus	33234	Ygbi	1	0	0	0	0	1	0	0	0	0	1	0
Oxyanthus subpunctatus	35009	Ygbi	1	0	0	0	0	1	0	0	0	0	1	0
Paramacrolobium coeruleum	1115	Ygbi	0									0	0	0
Paramacrolobium coeruleum	1129	Ygbi	0									0	0	1
Pentaclethra macrophylla	99	Luki	1	0	1	0	0	0	1	0	1	1	1	0
Pentaclethra macrophylla	1458	Luki	1	0	1	0	0	0	1	0	1	1	0	0
			2	0	0	1	0	0	1	0	1	0	1	0
Pentaclethra macrophylla	5153	Luki	0									0	0	0
Pentaclethra macrophylla	5244	Ygbi	0									0	0	0
Pericopsis elata	1151	Ygbi	0									0	0	0
Pericopsis elata	3613	Ygbi	1	1	0	0	0	0	1	0	1	1	0	0
			2	1	0	0	0	0	1	0	1	1	0	0
			3	0	0	0	0	0	1	0	1	0	1	0
Piptadeniastrum africanum	2425	Ygbi	0									0	0	1
Piptadeniastrum africanum	3618	Ygbi	0									0	0	1
Polyalthia suaveolens	818	Luki	1	0	0	0	0	0	0	0	1	0	1	0
Polyalthia suaveolens	1128	Ygbi	1	0	1	0	0	0	1	0	1	1	0	0
			2	0	1	0	0	0	1	0	1	1	0	0
			3	0	0	0	0	0	1	0	1	0	1	0
			4	0	0	0	0	0	1	0	1	0	1	0
Polyalthia suaveolens	5198	Ygbi	1	0	1	0	0	0	1	0	1	1	0	0
Polyalthia suaveolens	5199	Ygbi	1	0	1	0	0	0	1	0	1	1	0	0

			2	0	1	0	0	0	1	0	1	1	0	0
Polyalthia suaveolens	26318	Ygbi	1	0	1	0	0	0	1	0	1	1	0	0
			2	0	1	0	0	0	1	0	1	1	0	0
			3	0	1	0	0	0	1	0	1	1	0	0
Polyalthia suaveolens	32326	Luki	1	0	1	0	0	0	1	0	1	1	0	0
			2	0	1	0	0	0	1	0	1	1	0	0
Polyalthia suaveolens	32618	Luki	1	0	1	0	0	0	1	0	1	1	0	0
			2	0	1	0	0	0	1	0	1	1	0	0
Prioria balsamifera	1296	Luki	1	0	0	0	0	0	1	0	1	0	1	0
			2	0	0	0	0	0	1	0	1	0	1	0
Prioria balsamifera	1417	Luki	1	0	0	0	0	0	1	0	1	0	1	0
			2	0	0	0	1	1	1	0	1	1	0	0
Prioria balsamifera	5250	Ygbi	1	0	0	0	0	0	1	0	1	0	1	0
			2	0	0	0	0	1	0	0	1	0	1	0
Prioria oxyphylla	47	Luki	1	1	0	0	0	0	1	0	0	1	0	0
			2	0	0	0	0	0	1	0	1	0	1	0
Prioria oxyphylla	1109	Ygbi	1	0	0	0	0	0	1	0	1	0	1	0
			2	0	0	0	0	0	1	0	1	0	1	0
			3	0	0	0	0	0	1	0	1	0	1	0
Prioria oxyphylla	1285	Luki	1	0	0	0	0	0	1	0	1	0	1	0
			2	0	0	0	0	0	1	0	1	0	1	0
Prioria oxyphylla	1467	Luki	1	0	0	0	0	0	1	0	0	0	1	0
Psychotria cyanopharynx	33215	Ygbi	1	0	0	0	0	0	0	0	0	0	1	0
Psychotria cyanopharynx	33247	Ygbi	0									0	0	1
Psychotria cyanopharynx	33258	Ygbi	0									0	0	1
Psychotria cyanopharynx	35832	Ygbi	0									0	0	1
Psychotria cyanopharynx	38589	Ygbi	0									0	0	1
Pycnanthus angolensis	51	Luki	0									0	0	1
Pycnanthus angolensis	1437	Luki	0									0	0	1

Pycnanthus angolensis	32735	Ygbi	0									0	0	1
Pyrenacantha staudtii	32334	Luki	0									0	0	1
Pyrenacantha staudtii	34918	Ygbi	0									0	0	1
Pyrenacantha staudtii	35838	Ygbi	0									0	0	1
Ricinodendron heudelotii	122	Luki	1	0	0	1	0	0	0	0	0	0	1	0
Ricinodendron heudelotii	1119	Ygbi	0									0	0	0
Ricinodendron heudelotii	32733	Ygbi	1	0	1	0	0	0	0	0	0	1	0	0
Rinorea oblongifolia	33480	Ygbi	1	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	2	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	3	0	1	0	0	0	0	0	0	1	0	0
Rinorea oblongifolia	34992	Ygbi	1	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	2	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	3	0	1	0	0	0	0	0	0	1	0	0
Rinorea oblongifolia	68433	Ygbi	1	1	1	0	0	0	0	0	0	1	0	0
		Ygbi	2	1	1	0	0	0	0	0	0	1	0	0
		Ygbi	3	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	4	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	5	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	6	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	7	1	1	0	0	0	0	0	0	1	0	0
Rothmannia lateriflora	32312	Luki	0									0	0	1
Rothmannia lateriflora	33666	Ygbi	0									0	0	1
Rothmannia lateriflora	34783	Ygbi	1	0	0	0	0	0	1	1	0	0	1	0
		Ygbi	2	0	0	0	0	0	1	1	0	0	1	0
Rothmannia longiflora	32410	Luki	0									0	0	1
Rothmannia longiflora	32568	Luki	1	0	0	0	0	0	0	1	0	0	1	0
		Luki	2	0	0	0	0	0	0	1	0	0	1	0
Rothmannia longiflora	33023	Ygbi	1	1	0	0	0	1	0	0	0	1	0	0
		Ygbi	2	0	0	0	0	1	0	0	0	1	0	0

Rothmannia longiflora	33851	Ygbi	0									0	0	1
Rothmannia whitfieldii	32638	Luki	1	0	0	0	0	1	0	0	0	0	1	0
		Ygbi	2	0	0	0	0	1	0	0	0	0	1	0
		Ygbi	3	0	0	0	0	1	0	0	0	0	1	0
		Ygbi	4	0	0	0	0	1	0	0	0	0	1	0
Rothmannia whitfieldii	33122	Ygbi	1	0	0	0	0	1	0	0	0	0	1	0
Rothmannia whitfieldii	33281	Ygbi	1	0	0	0	0	1	0	0	0	0	1	0
Rothmannia whitfieldii	33561	Ygbi	0									0	0	1
Rourea coccinea var. viridis	32473	Luki	0									0	0	0
Rourea coccinea var. viridis	33072	Ygbi	1	0	0	1	0	0	0	0	0	0	1	0
		Ygbi	2	0	1	0	0	0	0	0	0	1	0	0
Rourea coccinea var. viridis	34899	Ygbi	1	0	0	1	0	0	0	0	0	0	1	0
Rourea thomsonii	32322	Luki	1	0	0	1	0	0	0	0	0	0	1	0
Rourea thomsonii	32447	Luki	1	0	1	0	0	0	0	0	0	1	0	0
Rourea thomsonii	32892	Ygbi	1	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	2	0	1	0	0	0	0	0	0	1	0	0
Rourea thomsonii	33238	Ygbi	1	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	2	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	3	0	1	0	0	0	0	0	0	1	0	0
Rourea thomsonii	33693	Ygbi	1	0	0	1	0	0	0	0	0	0	1	0
Rourea thomsonii	41264	Luki	1	0	1	0	0	0	0	0	0	1	0	0
Rourea thomsonii	41270	Luki	1	0	0	1	0	0	0	0	0	0	1	0
Salacia elegans	32847	Ygbi	0									0	0	1
Salacia elegans	33750	Ygbi	0									0	0	1
Salacia elegans	33819	Ygbi	0									0	0	1
Salacia staudtiana var. tshopoensis	32994	Ygbi	0									0	0	1
Salacia staudtiana var. tshopoensis	33028	Ygbi	0									0	0	0
Salacia staudtiana var. tshopoensis	33558	Ygbi	1	0	1	0	0	0	0	0	0	1	0	0
Salacia staudtiana var. tshopoensis	33771	Ygbi	0									0	0	1

Salacia staudtiana var. tshopoensis	38342	Ygbi	0										0	0	1
Salacia staudtiana var. tshopoensis	38421	Ygbi	0										0	0	1
Sorindeia juglandifolia	32340	Luki	0										0	0	0
Sorindeia juglandifolia	32374	Luki	0										0	0	1
Sorindeia juglandifolia	32768	Ygbi	0										0	0	1
Sorindeia juglandifolia	33120	Ygbi	0										0	0	0
Strombosia grandifolia	1291	Luki	0										0	0	1
Strombosia grandifolia	1456	Luki	1	0	0	1	0	0	0	0	0		0	1	0
Strombosia grandifolia	61106	Luki	0										0	0	0
Strombosia pustulata	1124	Ygbi	0										0	0	0
Strombosia pustulata	5200	Ygbi	0										0	0	1
Strombosia pustulata	5204	Ygbi	0										0	0	1
Strombosiopsis tetrandra	1122	Ygbi	0										0	0	1
Strombosiopsis tetrandra	2420	Ygbi	0										0	0	1
Strombosiopsis tetrandra	2426	Ygbi	0										0	0	1
Strophanthus sarmentosus	32437	Luki	1	0	1	0	0	0	1	0	0		1	0	0
		Luki	2	0	1	0	0	0	1	0	0		1	0	0
		Luki	3	0	1	0	0	0	1	0	0		1	0	0
Strophanthus sarmentosus	33619	Ygbi	1	0	1	0	0	0	1	0	0		1	0	0
Strophanthus sarmentosus	35010	Ygbi	1	0	1	0	0	0	1	0	0		1	0	0
		Ygbi	2	0	1	0	0	0	1	0	0		1	0	0
		Ygbi	3	0	1	0	0	0	1	0	0		1	0	0
Symphonia globulifera	929	Luki	0										0	0	1
Symphonia globulifera	1207	Luki	0										0	0	1
Symphonia globulifera	1425	Luki	0										0	0	1
Symphonia globulifera	2423	Ygbi	0										0	0	1
Synsepalum seretii	33507	Ygbi	1	0	0	0	0	0	1	1	0		0	1	0
		Ygbi	2	0	0	0	0	0	1	1	0		0	1	0
		Ygbi	3	0	0	0	0	0	1	1	0		0	1	0

		Ygbi	4	0	0	0	0	0	1	1	0	0	1	0
Synsepalum seretii	35169	Ygbi	1	0	1	0	0	0	1	1	0	1	0	0
		Ygbi	2	0	1	0	0	0	1	1	0	1	0	0
Synsepalum seretii	35754	Ygbi	0									0	0	1
Synsepalum seretii	38460	Ygbi	1	0	0	0	0	0	1	1	0	0	1	0
		Ygbi	1	0	0	0	0	0	1	1	0	0	1	0
Synsepalum subcordatum	1150	Ygbi	0									0	0	1
Synsepalum subcordatum	1167	Ygbi	1	0	0	1	0	0	1	1	0	0	1	0
Synsepalum subcordatum	2433	Ygbi	1	0	0	0	0	0	1	1	0	0	1	0
		Ygbi	2	0	0	0	0	0	1	1	0	0	1	0
		Ygbi	3	0	0	0	0	0	1	1	0	0	1	0
Synsepalum subcordatum	34892	Ygbi	1	0	1	0	0	0	1	0	0	1	0	0
		Ygbi	2	0	1	0	0	0	1	1	0	1	1	0
		Ygbi	3	0	0	0	0	0	1	1	0	0	1	0
Tricalysia pallens	33007	Ygbi	1	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	2	0	0	0	0	1	1	0	0	0	1	0
Tricalysia pallens	33157	Ygbi	0									0	0	0
Tricalysia pallens	38420	Ygbi	1	0	1	0	0	0	0	0	0	1	0	0
Trichilia gilgiana	32350	Luki	1	0	0	0	0	0	0	1	0	0	1	0
		Luki	2	0	0	0	0	0	0	1	0	0	1	0
Trichilia gilgiana	32658	Luki	1	1	0	0	0	0	1	0	0	1	0	0
		Luki	2	0	1	0	0	0	1	0	0	1	0	0
Trichilia prieuriana	104	Luki	1	0	1	0	0	0	1	1	0	1	0	0
		Luki	2	0	1	0	0	0	1	1	0	1	0	0
		Luki	3	0	1	0	0	0	1	0	0	1	0	0
		Luki	4	0	1	0	0	0	1	0	0	1	0	0
Trichilia prieuriana	32299	Luki	0									0	0	1
Trichilia prieuriana	32413	Luki	1	0	1	0	0	0	1	0	0	1	0	0
Trichilia prieuriana	32894	Ygbi	0									0	0	1

Trichilia rubescens	33105	Ygbi	1	0	1	0	0	0	1	1	0	1	0	0
		Ygbi	2	0	1	0	0	0	1	1	0	1	0	0
		Ygbi	3	0	1	0	0	0	1	1	0	1	0	0
		Ygbi	4	0	0	0	0	0	1	1	0	0	1	0
		Ygbi	5	0	0	0	0	0	1	1	0	0	1	0
Trichilia rubescens	33246	Ygbi	1	0	0	1	0	0	1	0	0	0	1	0
		Ygbi	2	0	0	1	0	0	1	0	0	0	1	0
Trichilia rubescens	68406	Ygbi	1	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	2	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	3	0	0	0	0	0	1	1	0	0	1	0
Tridesmostemon claessensii	5235	Ygbi	1	0	0	0	0	0	0	1	0	0	1	0
		Ygbi	2	0	0	0	0	0	0	1	0	0	1	0
		Ygbi	3	0	0	0	0	0	0	1	0	0	1	0
		Ygbi	4	0	0	0	0	0	0	1	0	0	1	0
Tridesmostemon claessensii	5237	Ygbi	1	0	0	0	0	0	0	1	0	0	1	0
Tridesmostemon claessensii	32739	Ygbi	0									0	0	0
Tridesmostemon claessensii	32951	Ygbi	1	0	1	0	0	0	1	0	0	1	0	0
		Ygbi	2	0	1	0	0	0	1	0	0	1	0	0
Trilepisium madagascariense	32949	Ygbi	1	0	0	0	0	0	1	1	0	0	1	0
Uvariopsis congolana	26319	Ygbi	1	1	0	0	0	0	0	0	1	1	0	0
		Ygbi	2	1	1	0	0	0	0	0	1	1	0	0
		Ygbi	3	1	0	0	0	0	0	0	1	1	0	0
		Ygbi	4	0	0	0	0	0	1	0	1	1	0	0
		Ygbi	5	1	0	0	0	0	1	0	1	1	0	0
		Ygbi	6	1	1	0	0	0	1	0	1	1	0	0
		Ygbi	7	0	0	0	0	0	1	0	1	1	0	0
Uvariopsis congolana	34919	Ygbi	1	0	0	0	0	0	0	1	1	0	1	0
		Ygbi	2	0	0	0	0	0	0	1	1	0	1	0
Uvariopsis congolana	38374	Ygbi	0									0	0	1

Vitex welwitschii	33431	Ygbi	1	0	1	0	0	0	0	0	0	1	0	0
Vitex welwitschii	35796	Ygbi	1	1	0	0	0	1	0	0	0	1	0	0
		Ygbi	2	1	1	0	0	1	0	0	0	1	0	0
		Ygbi	3	1	1	0	0	1	0	0	0	1	0	0
		Ygbi	4	1	1	0	0	1	0	0	0	1	0	0
Vitex welwitschii	35900	Ygbi	0									0	0	1
Voacanga bracteata	32978	Ygbi	1	0	0	0	0	0	0	0	0	0	1	0
Voacanga bracteata	33244	Ygbi	0									0	0	1
Voacanga bracteata	35052	Ygbi	0									0	0	1
Voacanga bracteata	35729	Ygbi	0									0	0	1
Zanthoxylum gilletii	77	Luki	1	0	0	0	0	0	1	0	0	0	1	0
		Luki	2	0	0	0	0	0	1	0	0	0	1	0
Zanthoxylum gilletii	5247	Ygbi	0									0	0	1
Zanthoxylum gilletii	33442	Ygbi	0									0	0	1
Zanthoxylum gilletii	33455	Ygbi	0									0	0	1
Zanthoxylum lemairei	33420	Ygbi	0									0	0	1
Zanthoxylum lemairei	33617	Ygbi	1	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	2	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	3	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	4	0	1	0	0	1	0	0	0	1	0	0
		Ygbi	5	0	1	0	0	1	0	0	0	1	0	0
		Ygbi	6	0	1	0	0	0	0	0	0	1	0	0
Zanthoxylum lemairei	35793	Ygbi	1	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	2	0	1	0	0	1	1	0	0	1	0	0
		Ygbi	3	0	1	0	0	0	0	0	0	1	0	0
Zanthoxylum lemairei	38370	Ygbi	1	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	2	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	3	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	4	0	1	0	0	1	0	0	0	1	0	0

		Ygbi	5	0	1	0	0	0	0	0	0	1	0	0
		Ygbi	6	0	1	0	0	0	0	0	0	1	0	0
Zanthoxylum rubescens	26323	Ygbi	1	0	0	0	0	1	1	0	0	1	0	0
		Ygbi	2	0	0	0	0	1	1	0	0	1	0	0
		Ygbi	3	0	0	0	0	1	1	0	0	1	0	0
Zanthoxylum rubescens	32917	Ygbi	1	0	1	0	0	0	1	0	1	1	0	0
		Ygbi	2	0	1	0	0	1	1	0	1	1	0	0
		Ygbi	3	0	1	0	0	1	1	0	1	1	0	0
		Ygbi	4	0	1	0	0	1	1	0	1	1	0	0
		Ygbi	5	0	1	0	0	1	1	0	1	1	0	0
		Ygbi	6	0	1	0	0	1	1	0	1	1	0	0
		Ygbi	7	0	1	0	0	1	1	0	1	1	0	0
Zanthoxylum rubescens	33805	Ygbi	1	0	1	0	0	1	1	0	1	1	0	0

Annex 1: List of species, their TW-number, site, number of rings observed, the anatomical features defining ring boundaries and growth-ring distinctness.

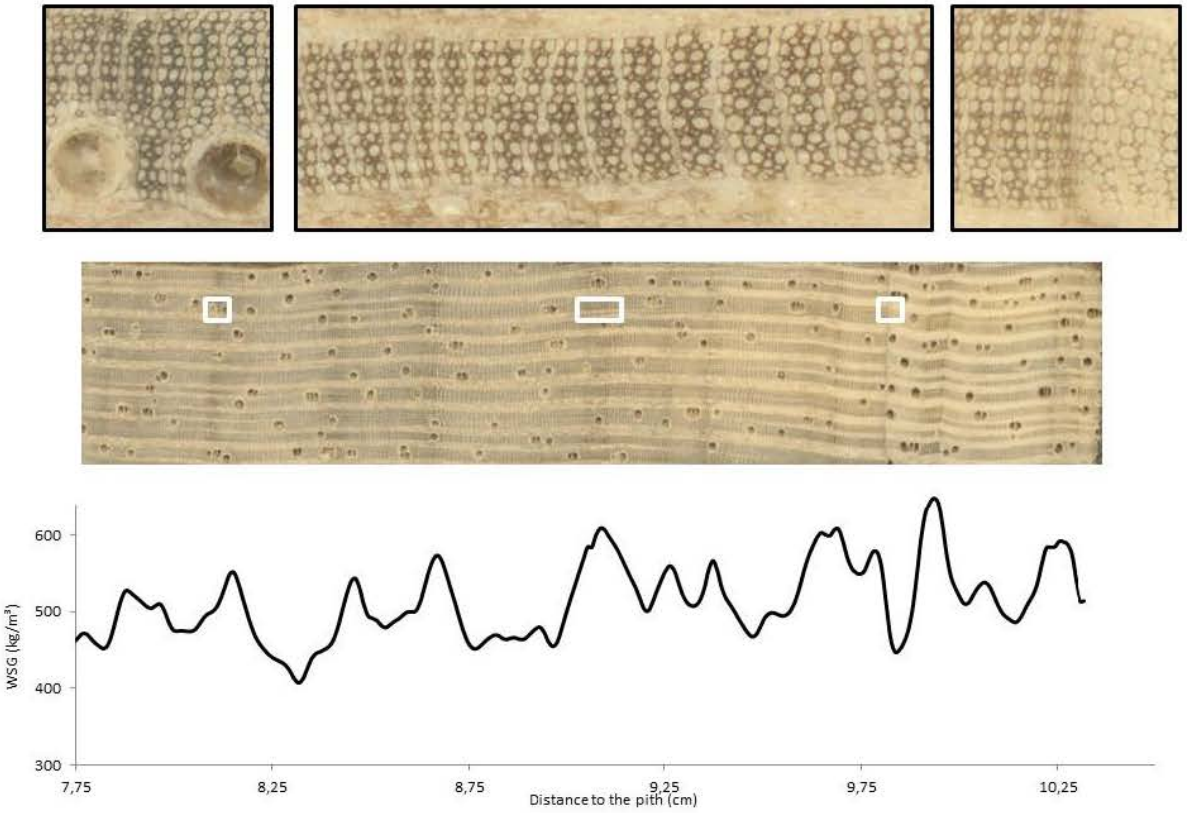
AC_FW: abrupt change in fibre wall thickness; AC_FD: abrupt change in fibre diameter; GC_FD: gradual change in fibre diameter; GC_VD: gradual change in vessel diameter; VDV: vessel density variation; MP: marginal parenchyma; PDV: parenchyma density variation; DR: distended rays

D: distinct; I: indistinct and A: absent.

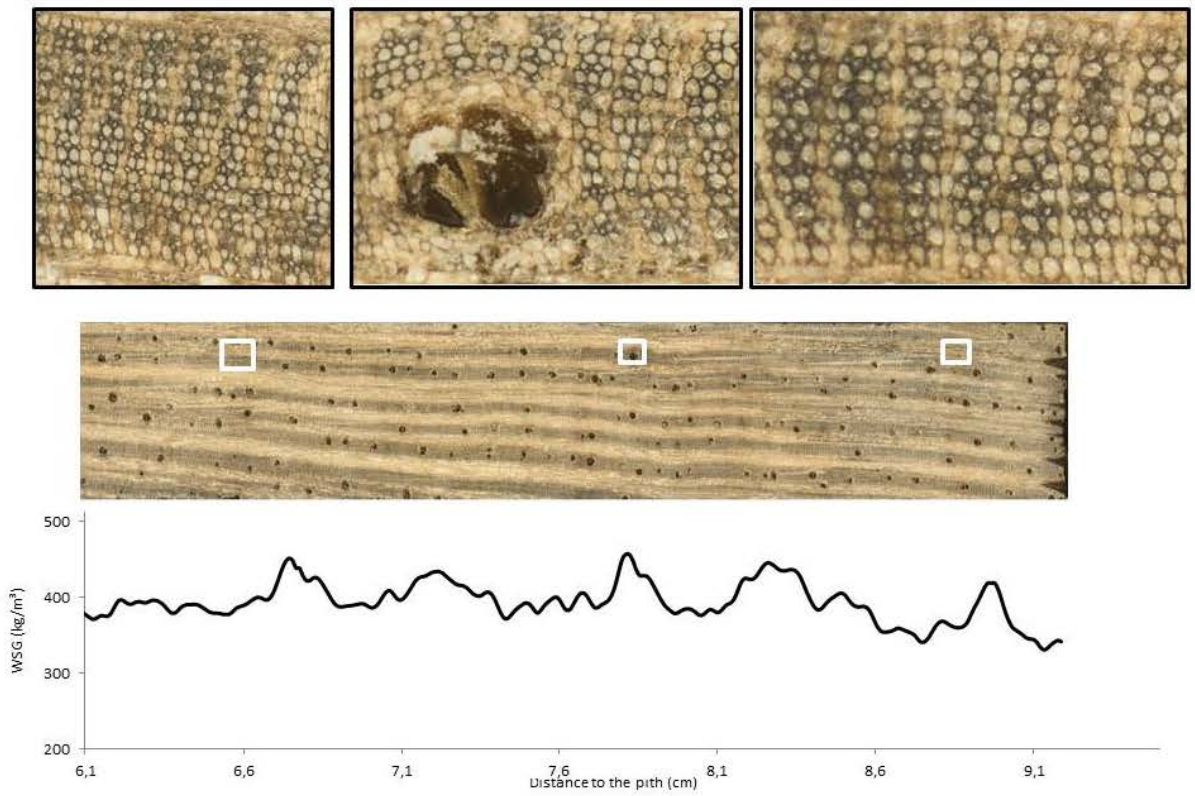
Annex 3

Annex 3: Additional illustrations for Paper 3

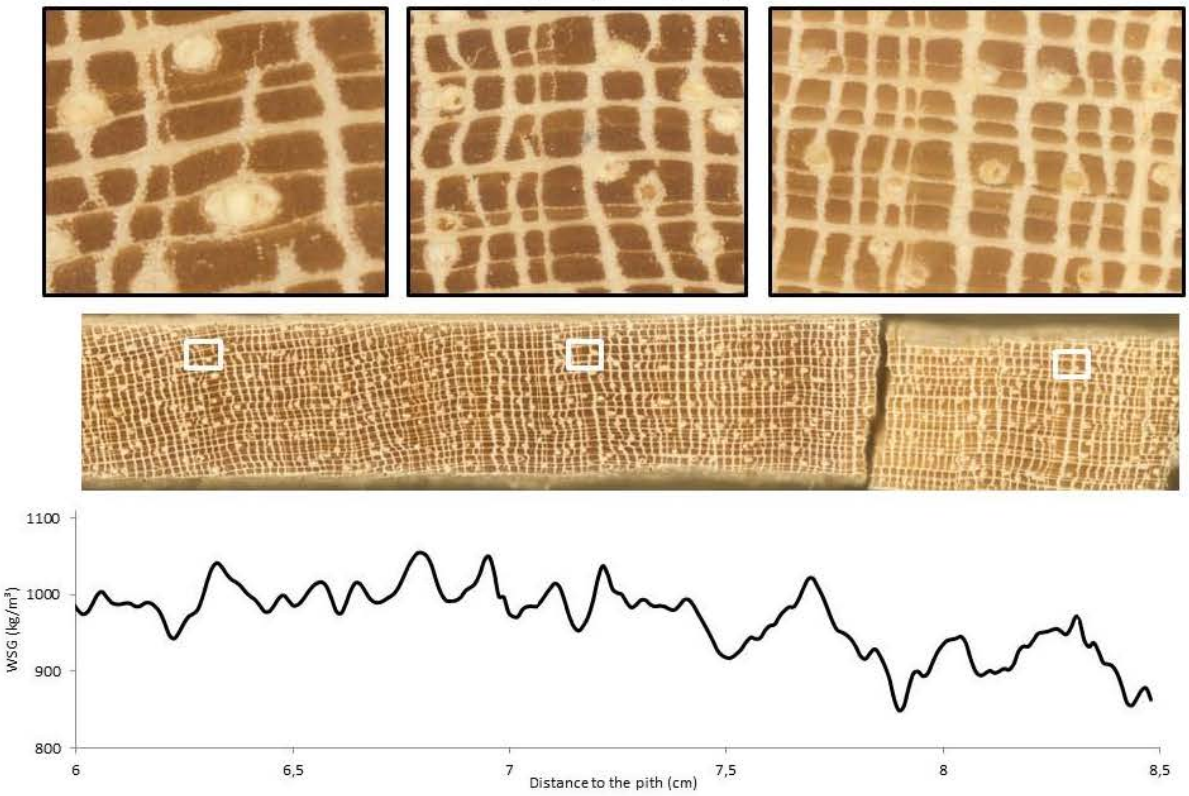
Anonidium manii (Malebo)



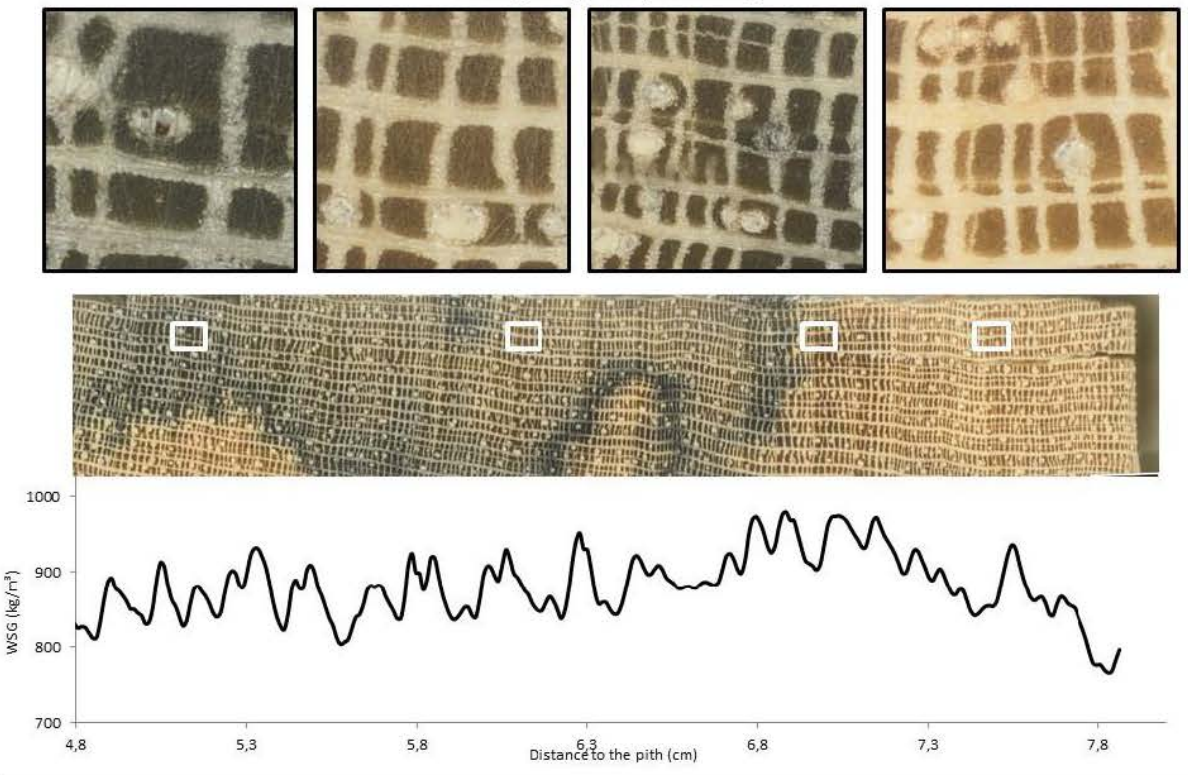
Anonidium manii (Yoko)



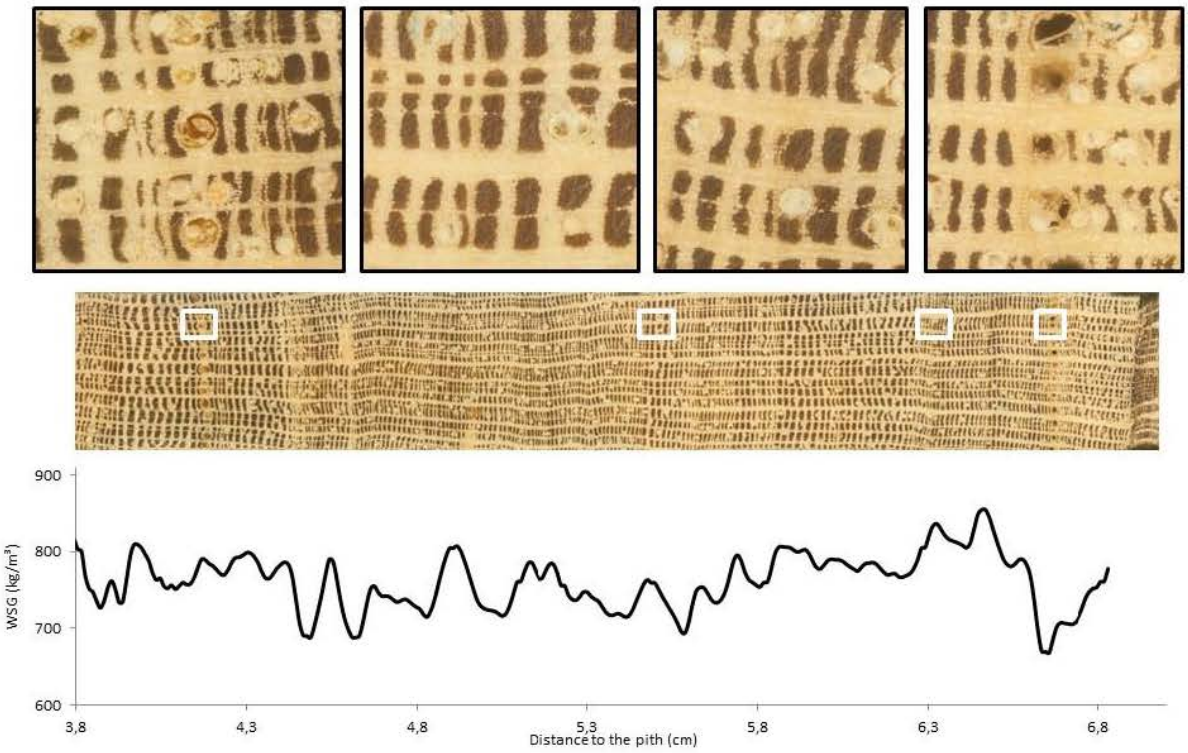
Cola griseiflora (Luki)



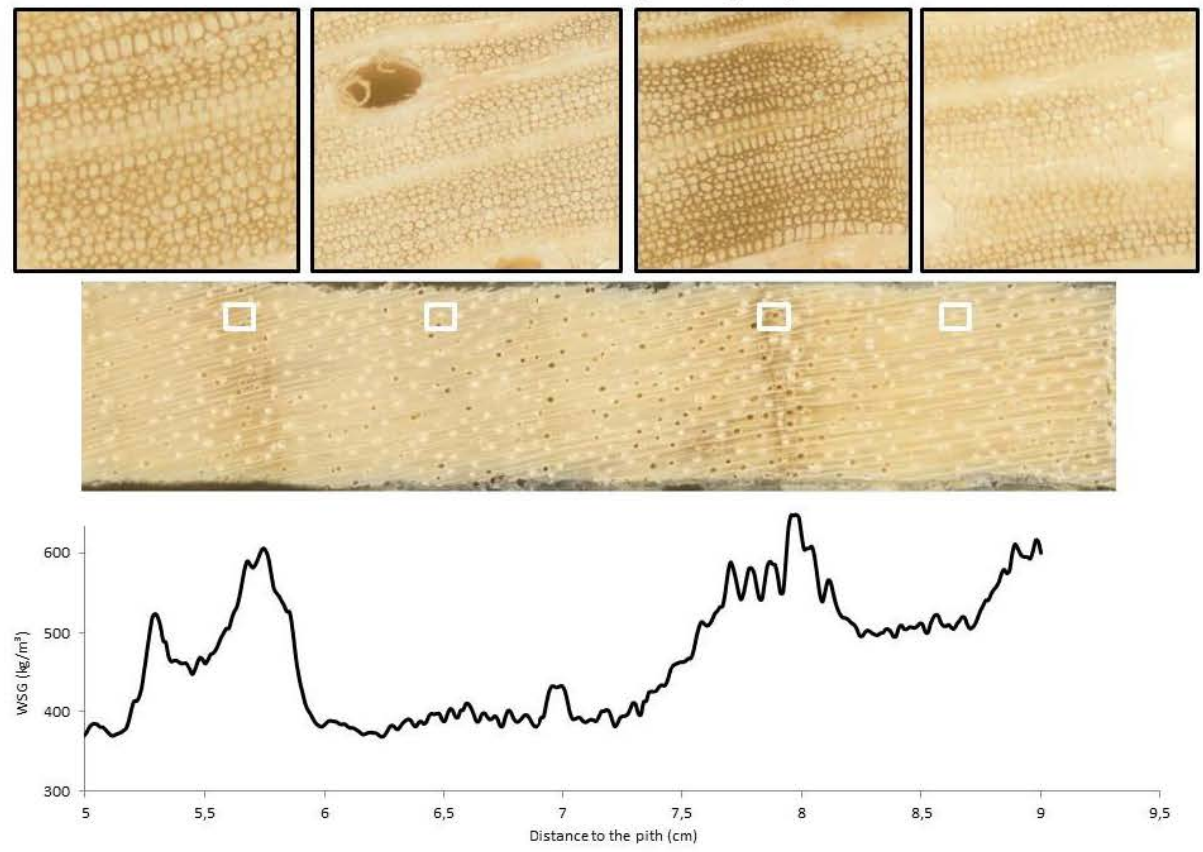
Cola griseiflora (Malebo)



Cola griseiflora (Yoko)

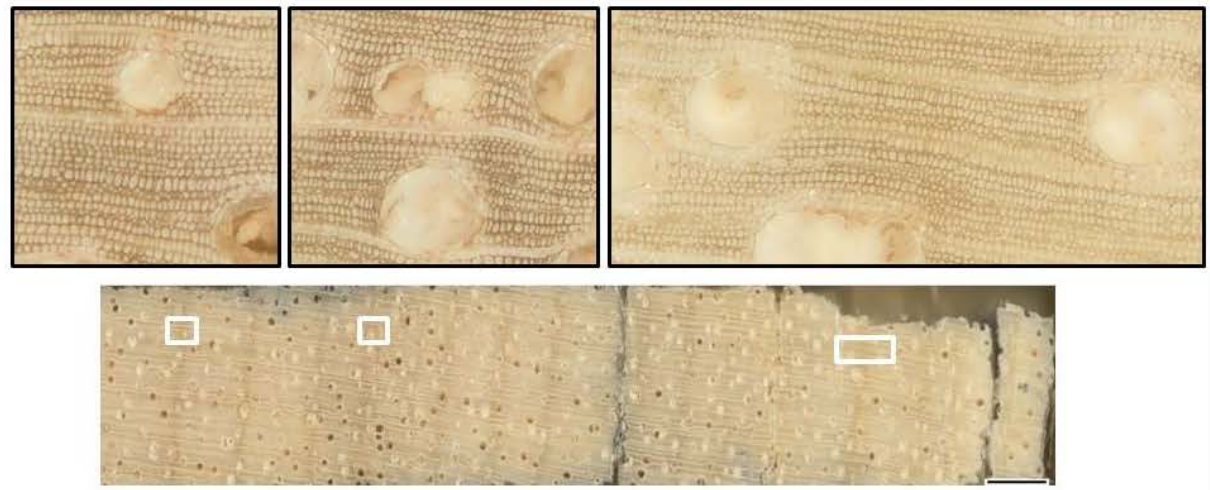


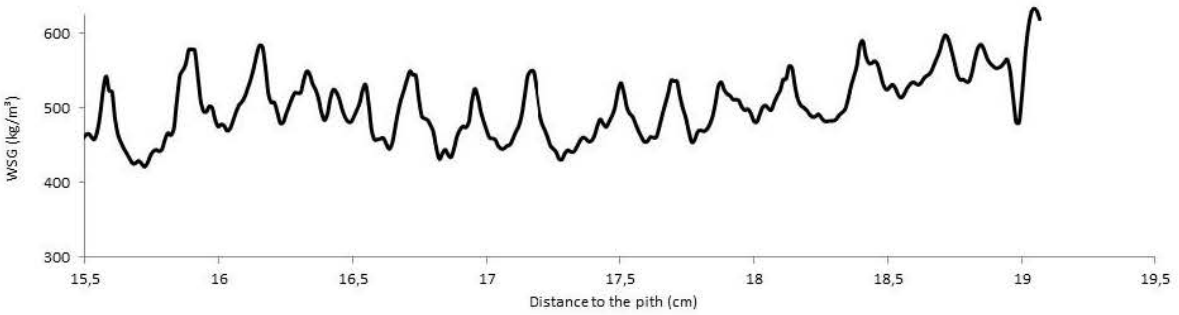
Canarium schweinfurthii (Luki)



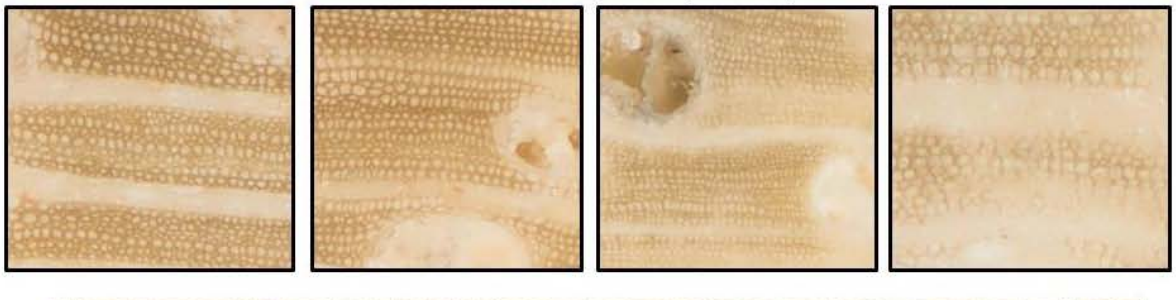
Annex 3

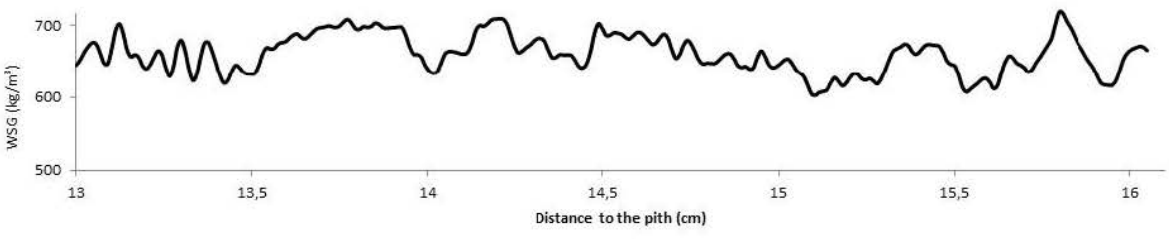
Canarium schweinfurthii (Yoko)



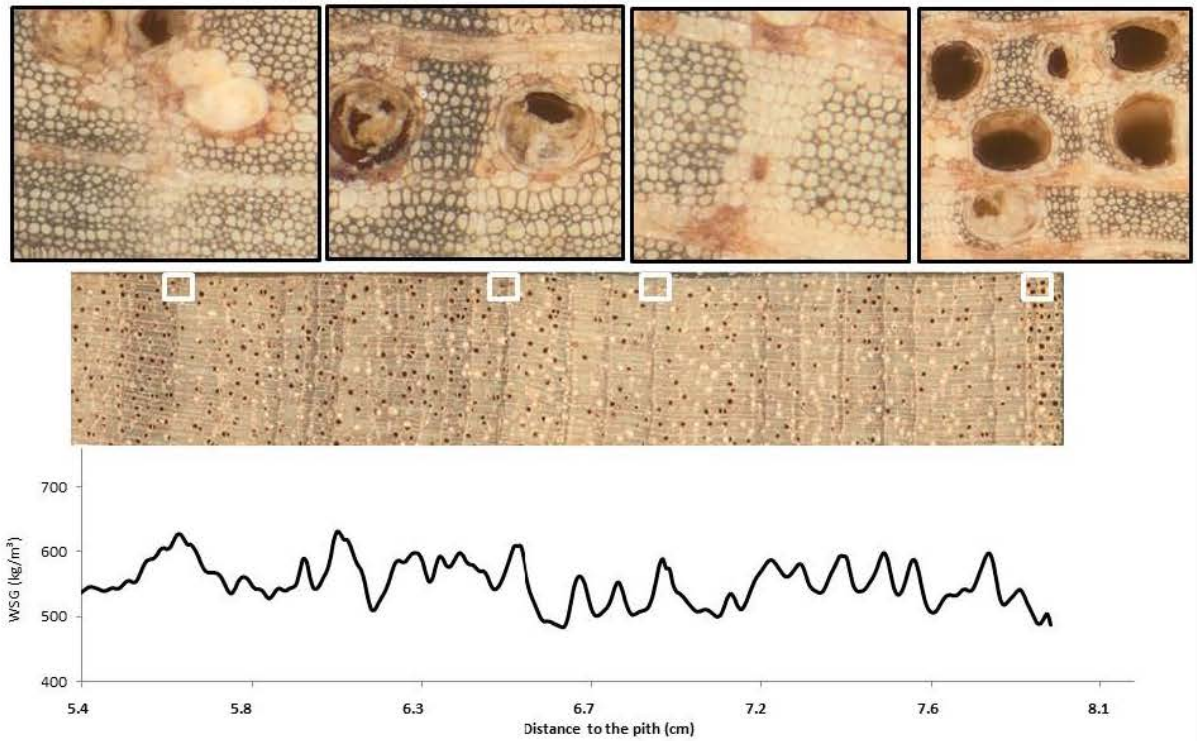


Canarium schweinfurthii (Malebo)

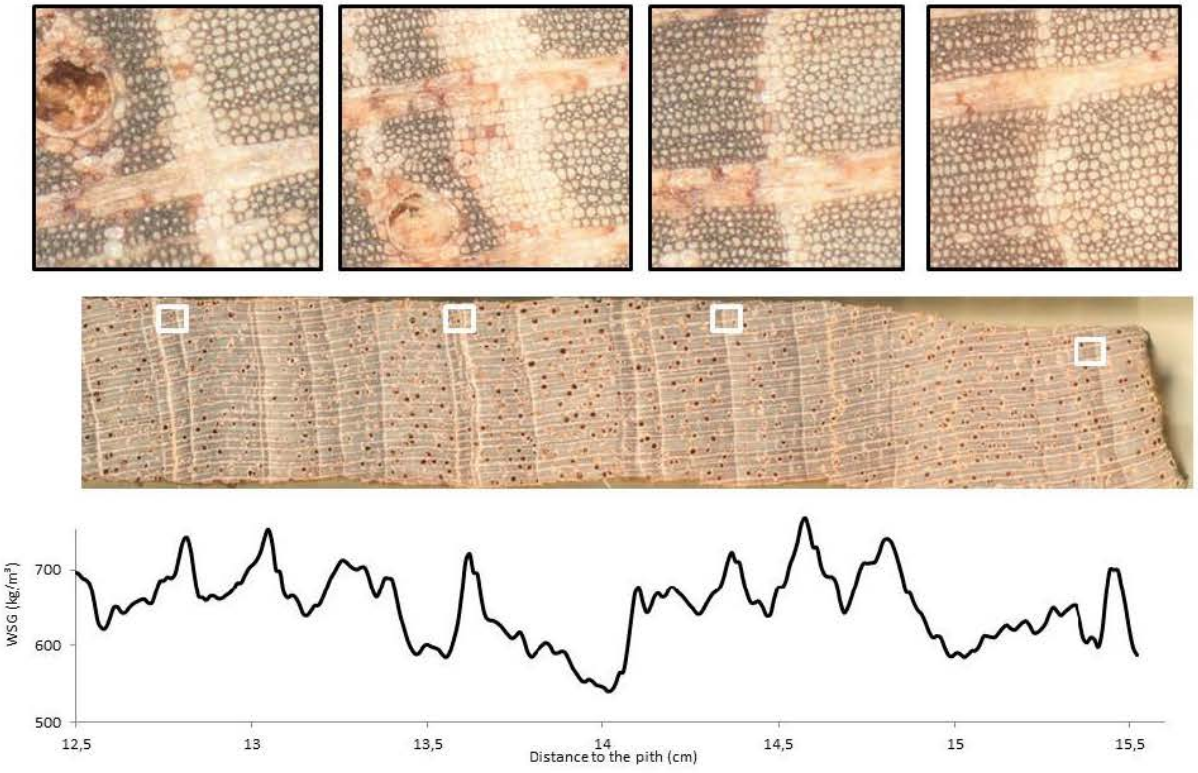




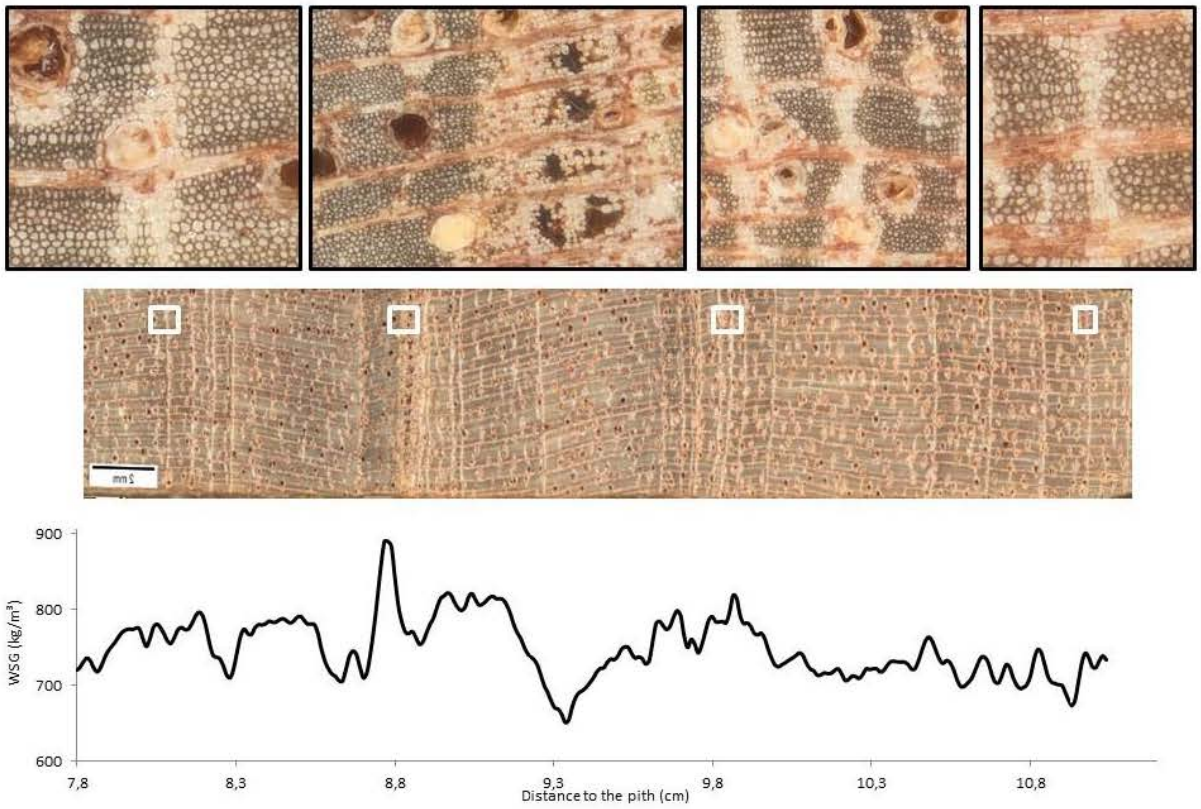
Entandrophragma angolense (Luki)



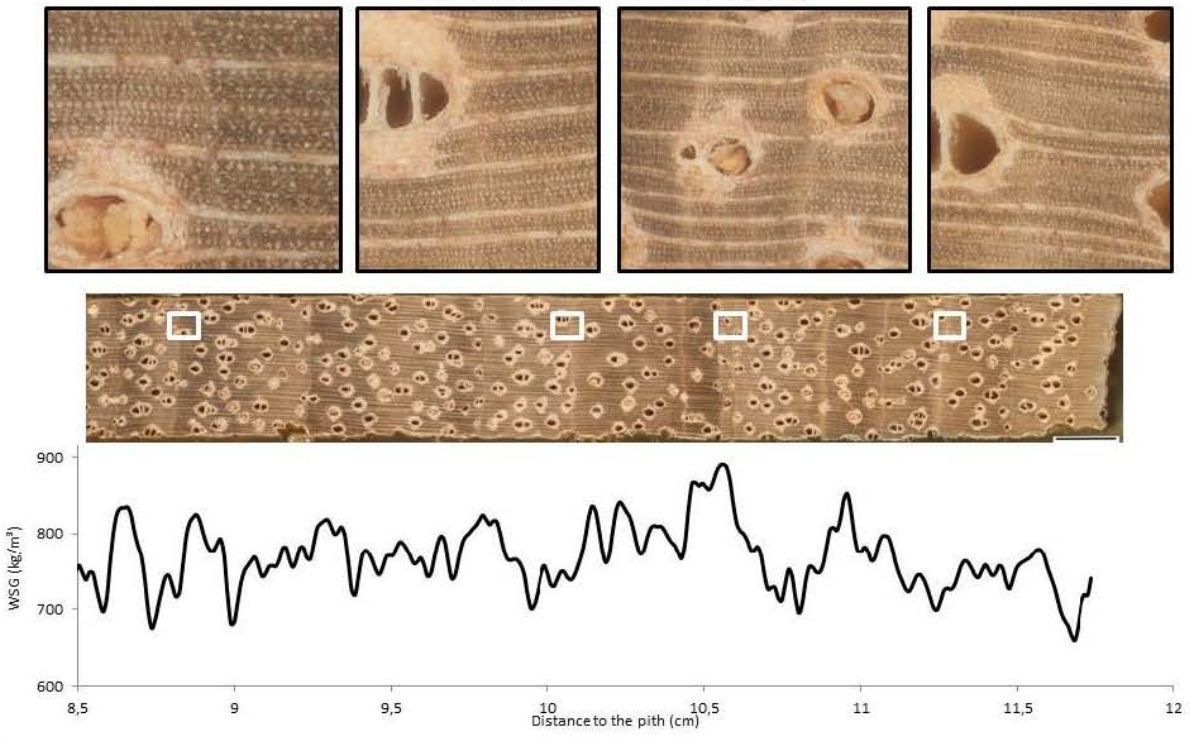
Entandrophragma angolense (Malebo)



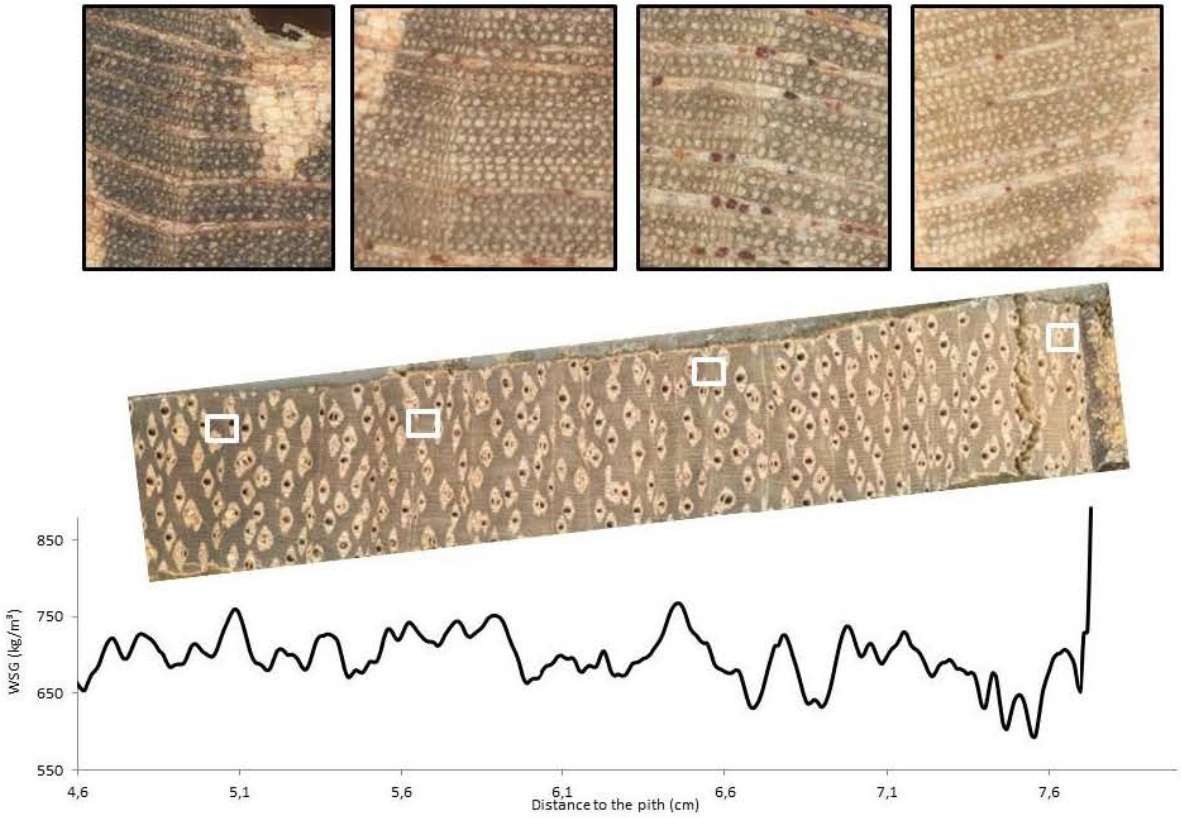
Entandrophragma angolense (Yoko)



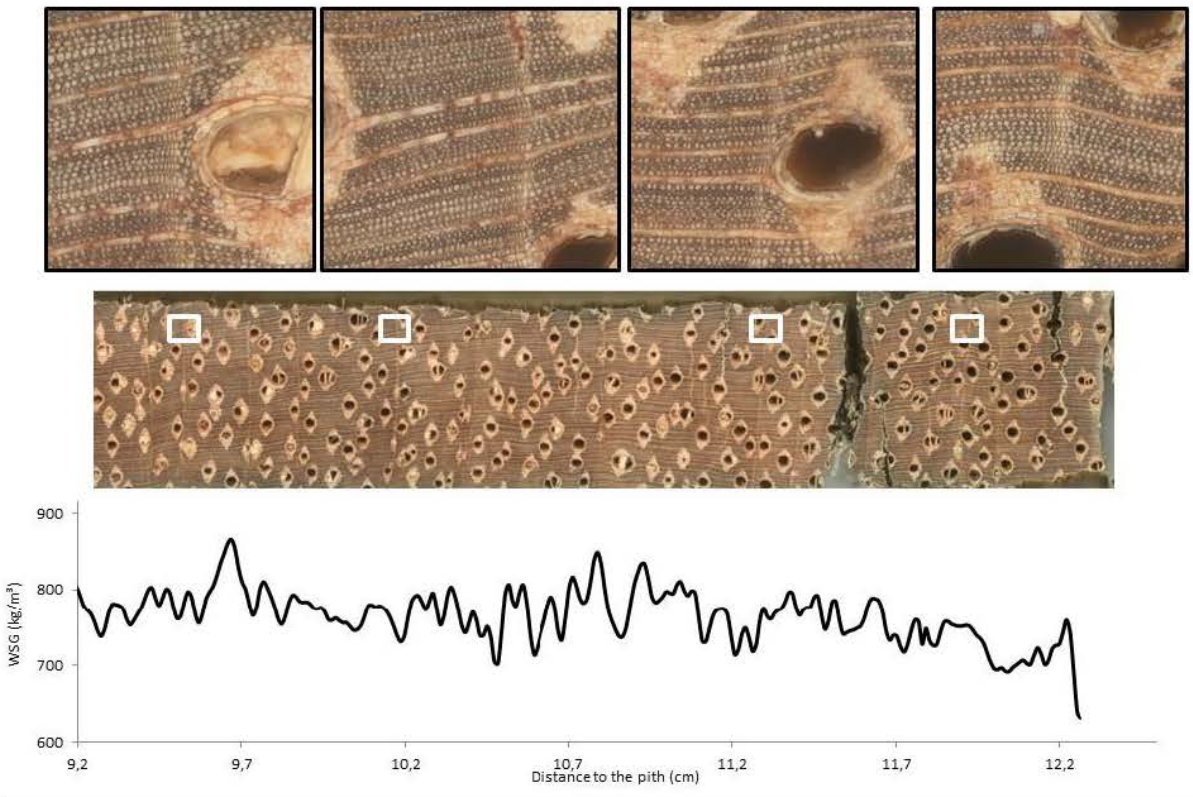
Gilbertiodendron dewevrei (Luki)



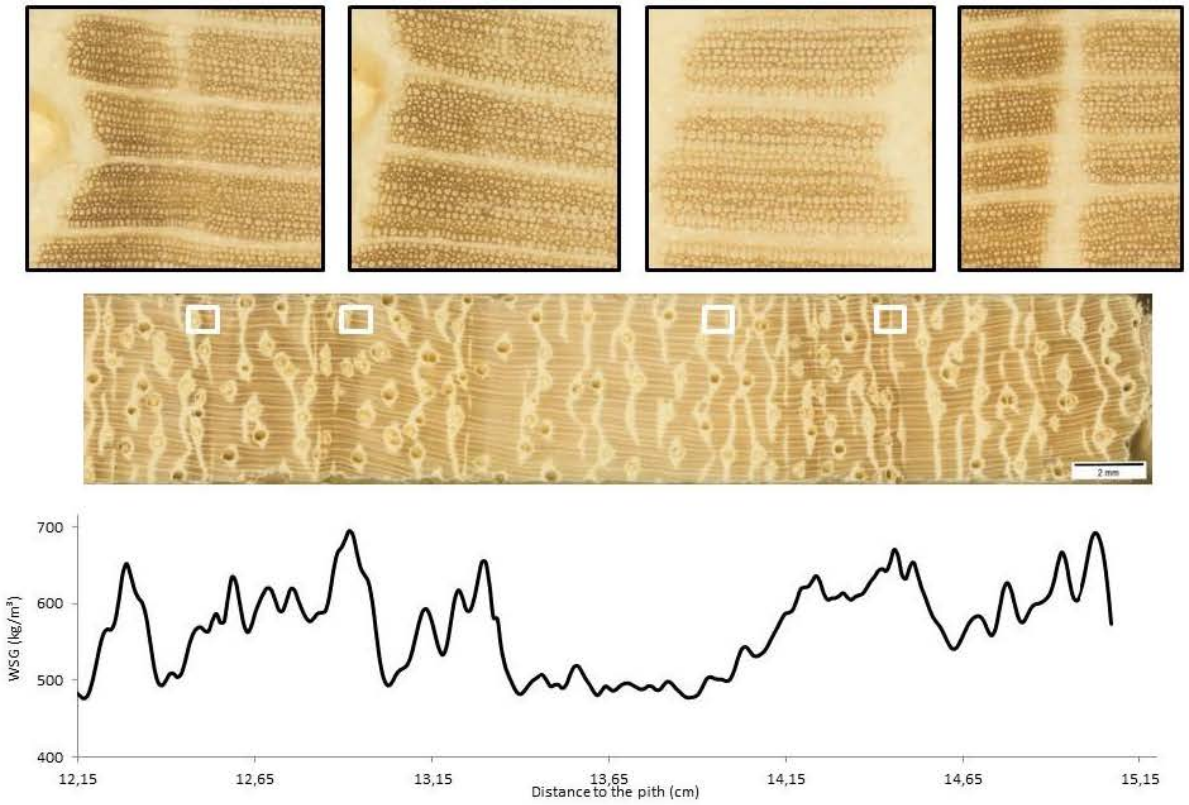
Gilbertiodendron dewevrei (Malebo)



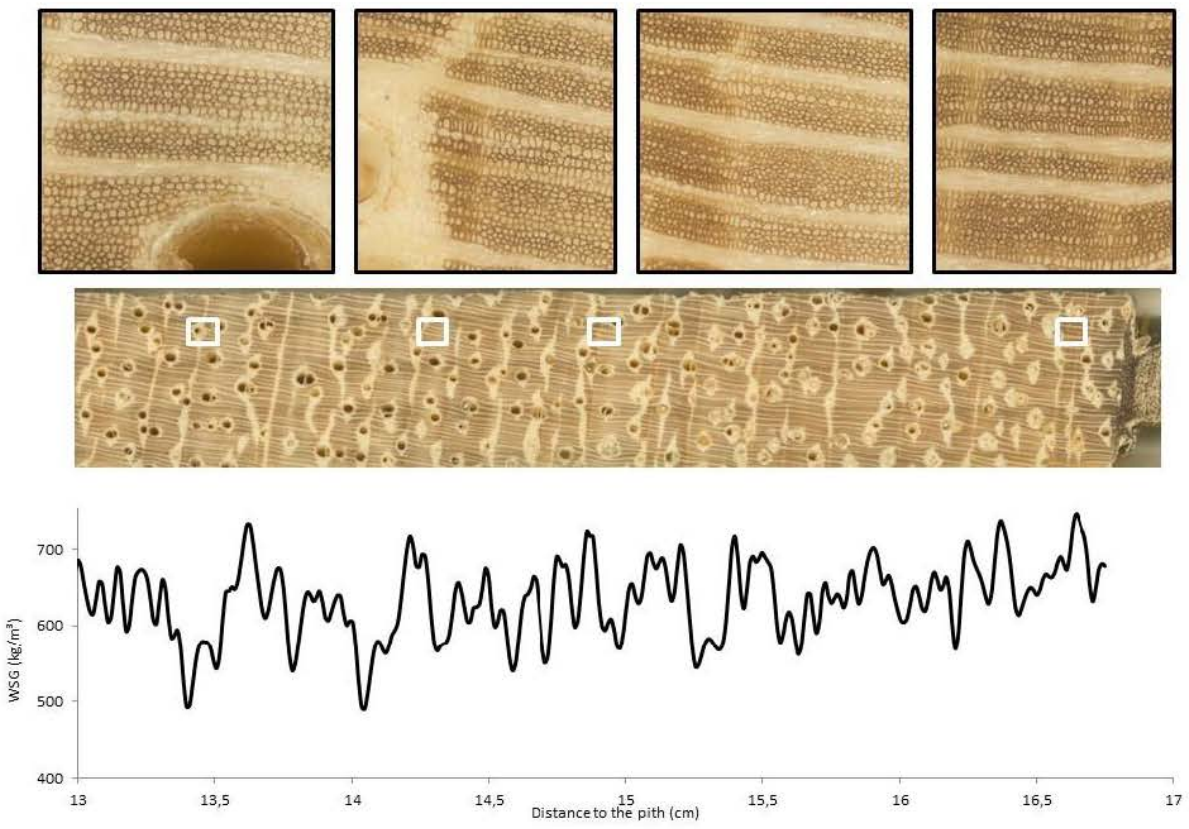
Gilbertiodendron dewevrei (Yoko)



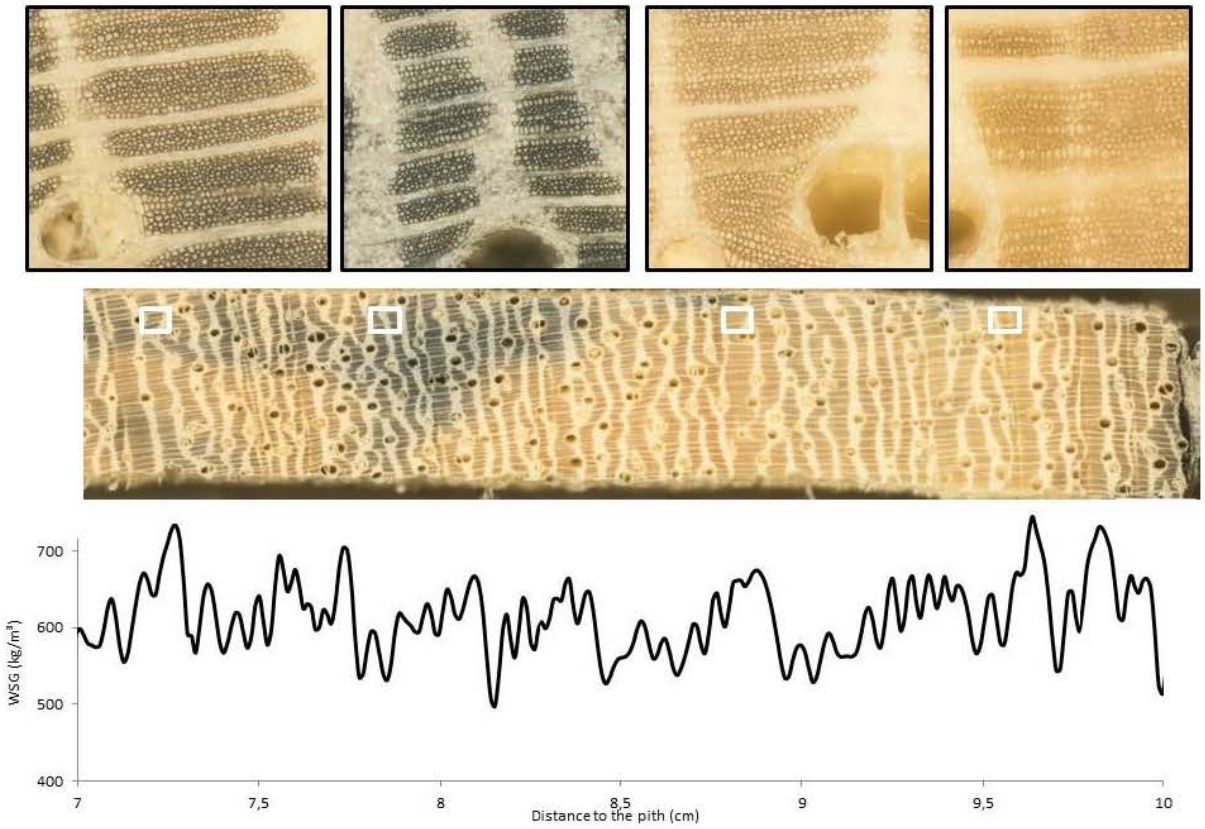
Milicia excelsa (Luki)



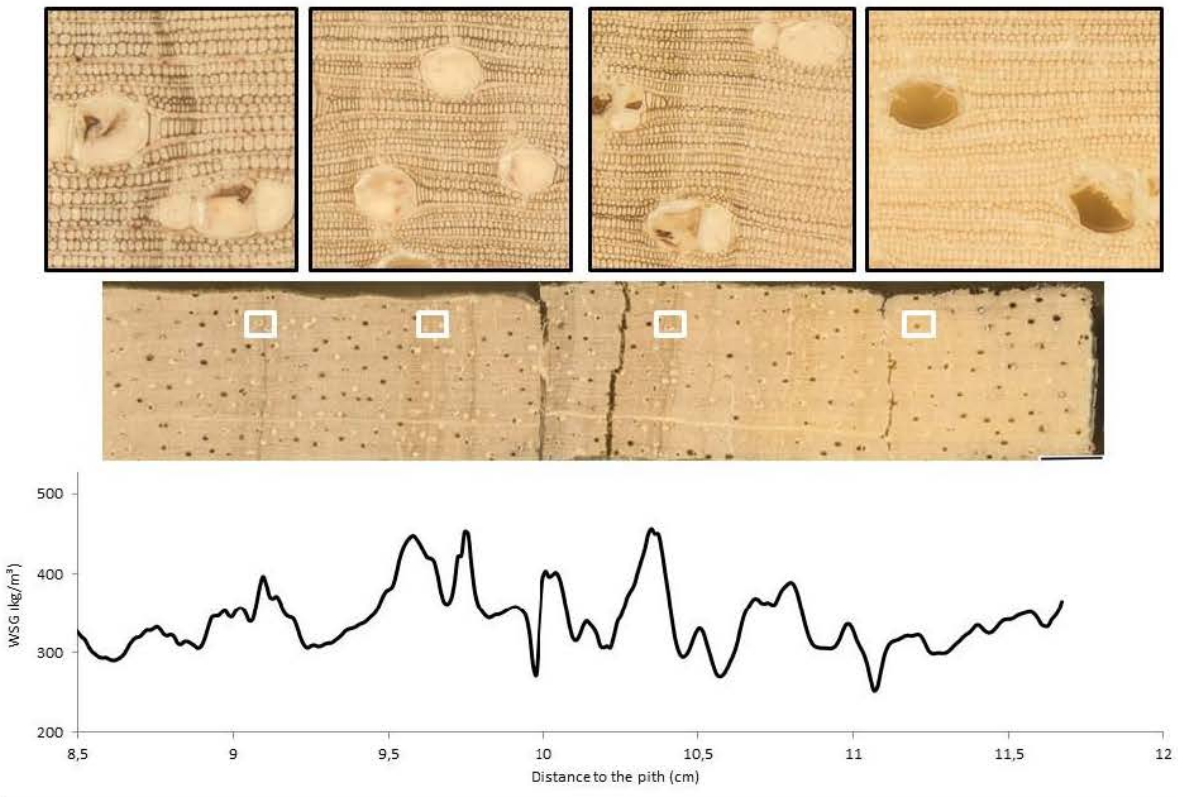
Milicia excelsa (Malebo)



Milicia excelsa (Yoko)

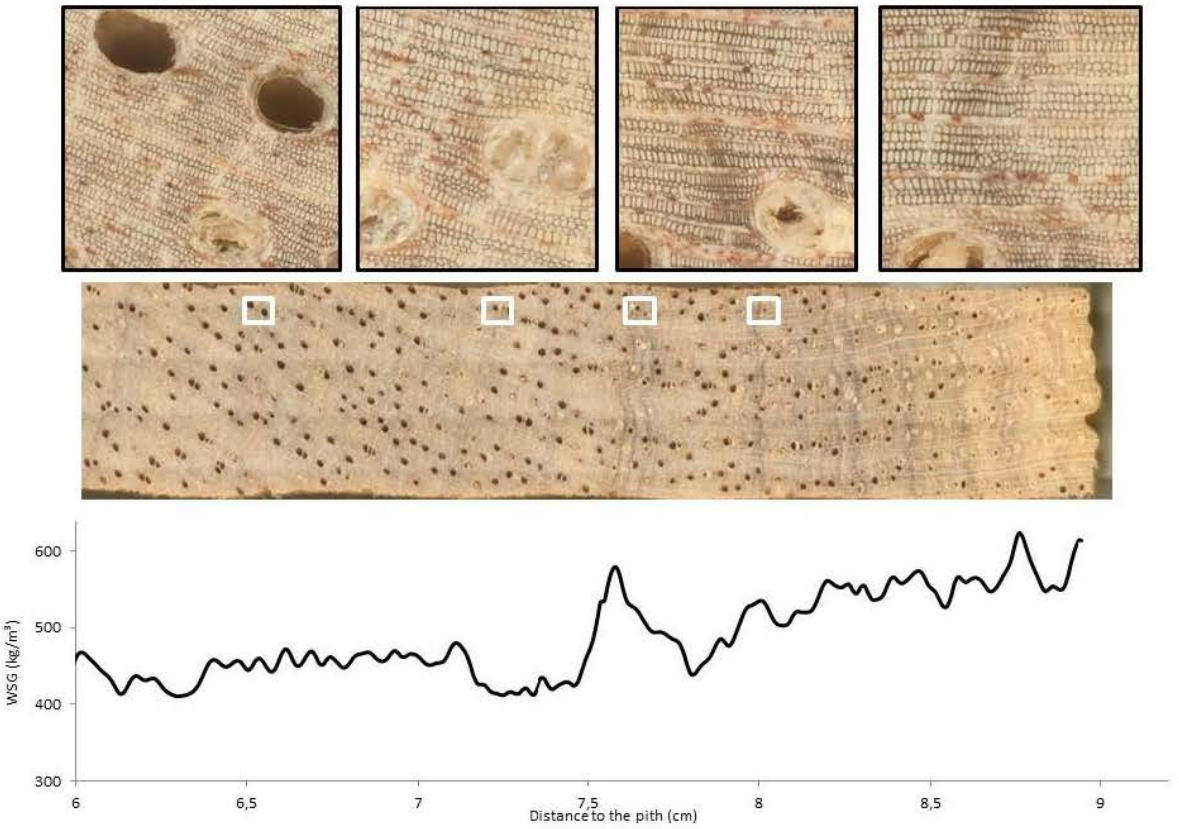


Macaranga spinosa (Luki)

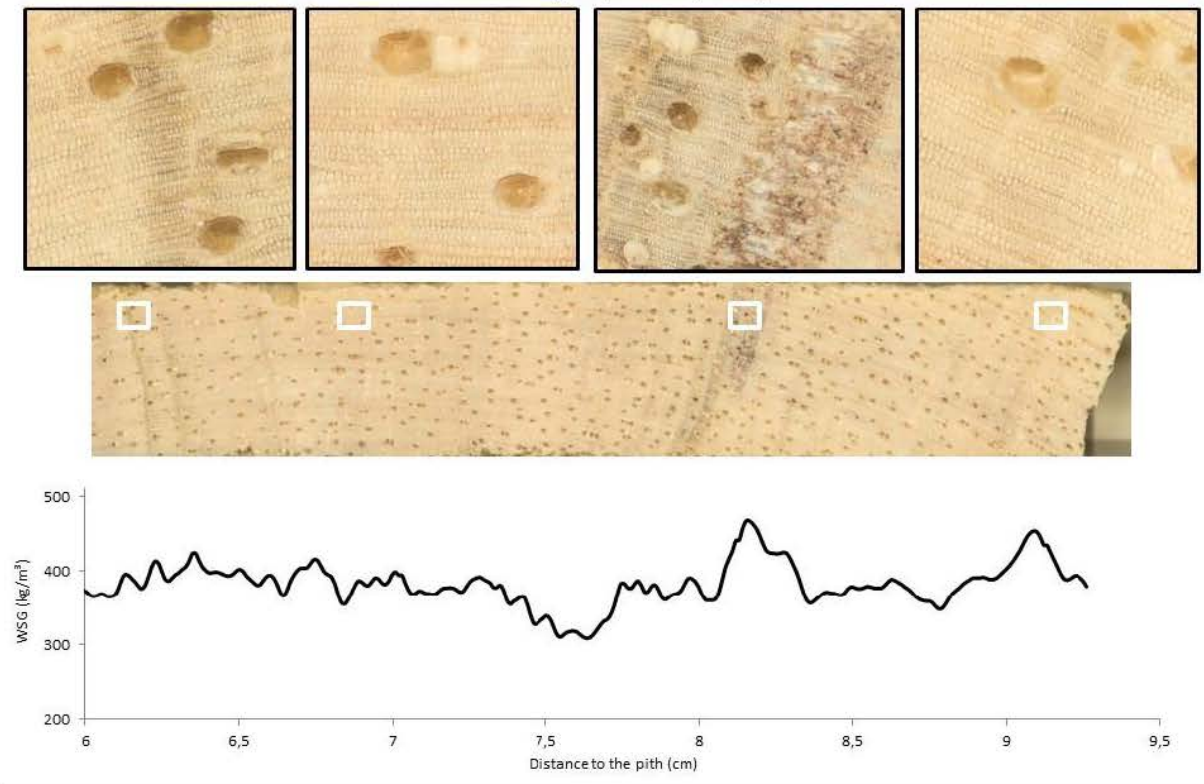


Annex 3

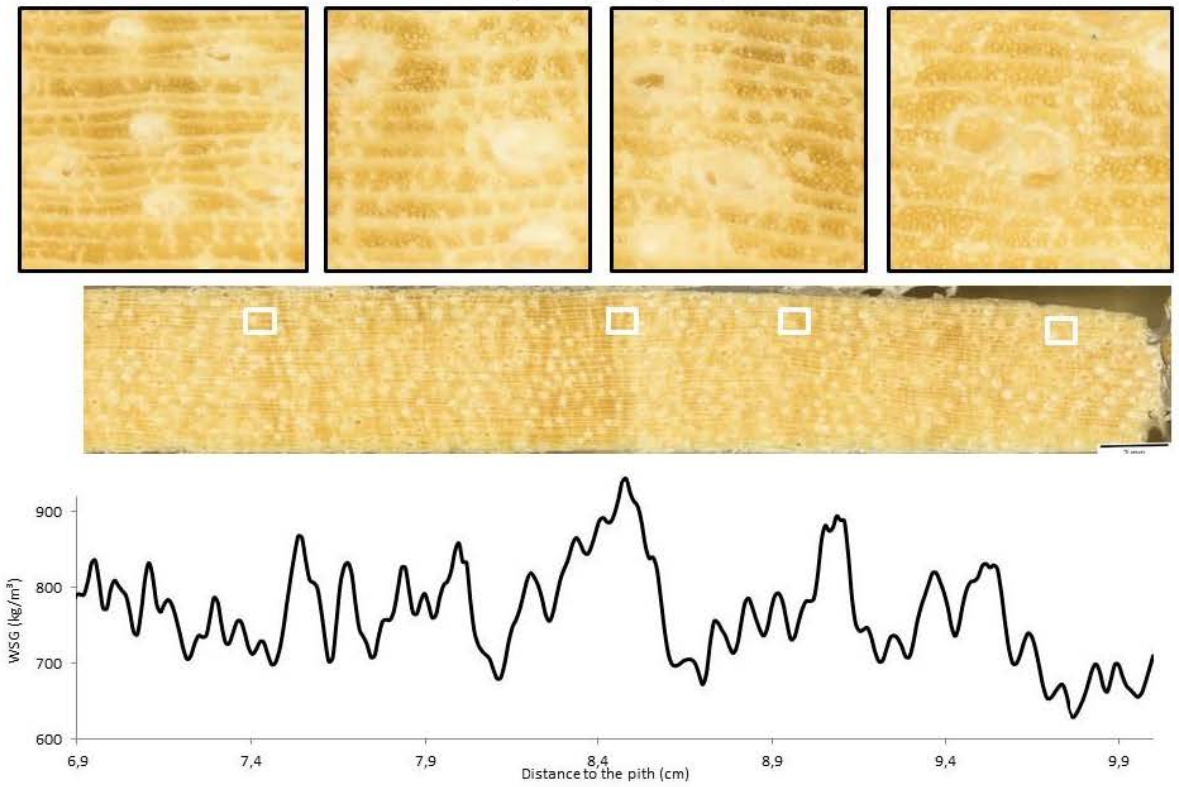
Macaranga spinosa (Malebo)



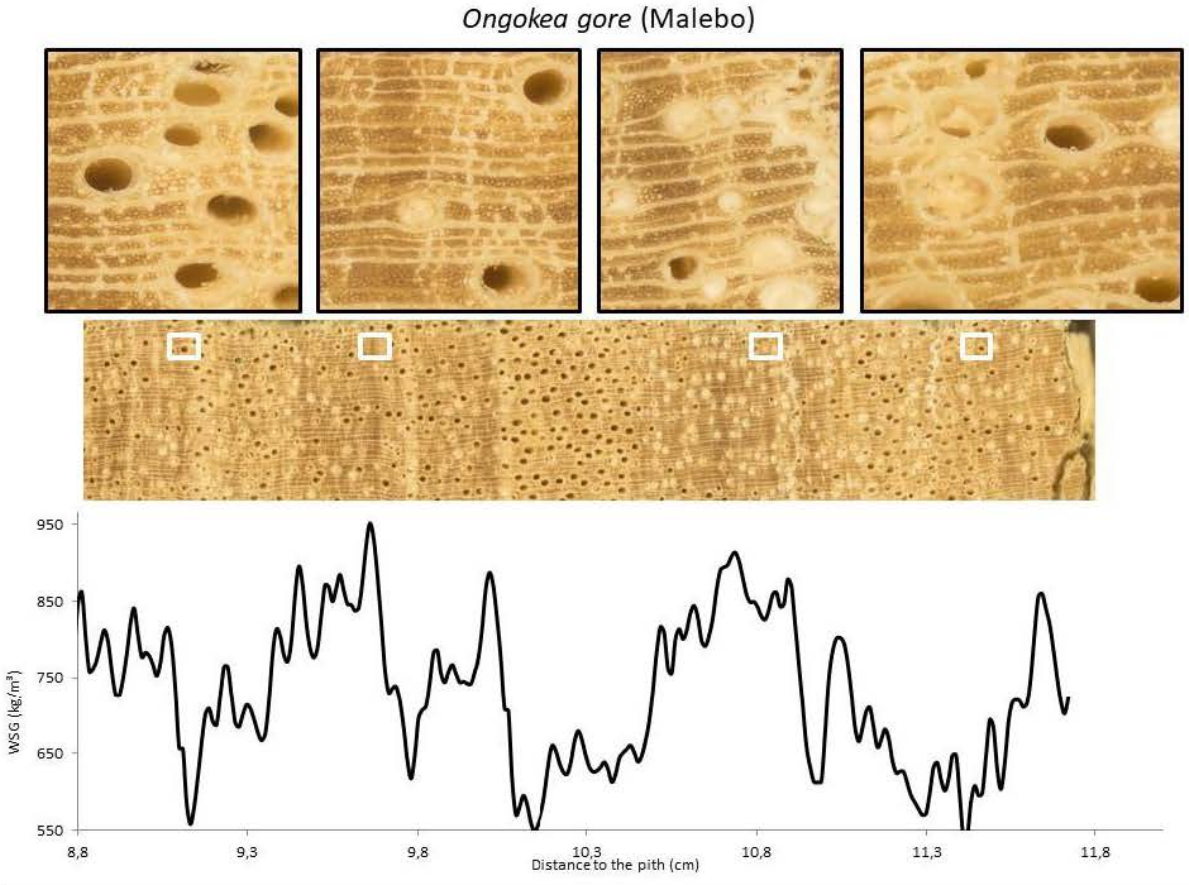
Macaranga spinosa (Yoko)



Ongokea gore (Luki)

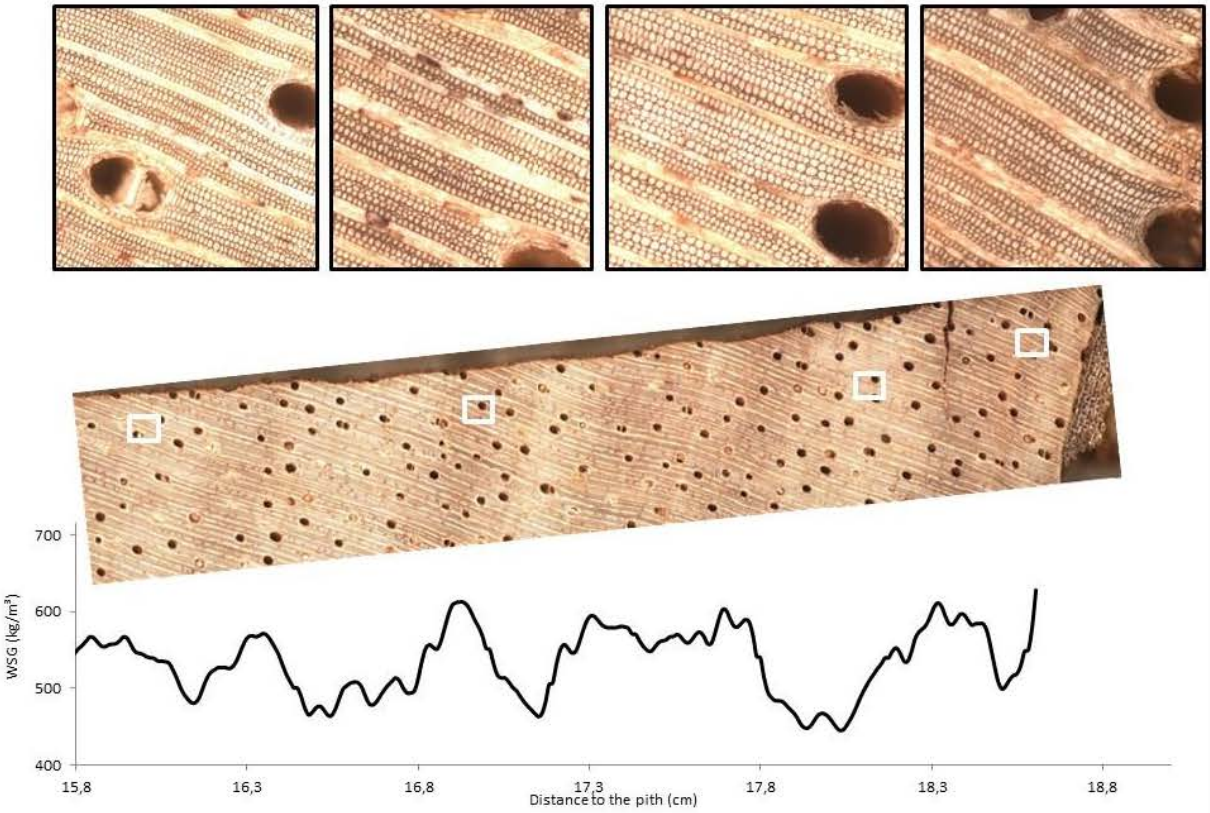


Ongokea gore (Malebo)

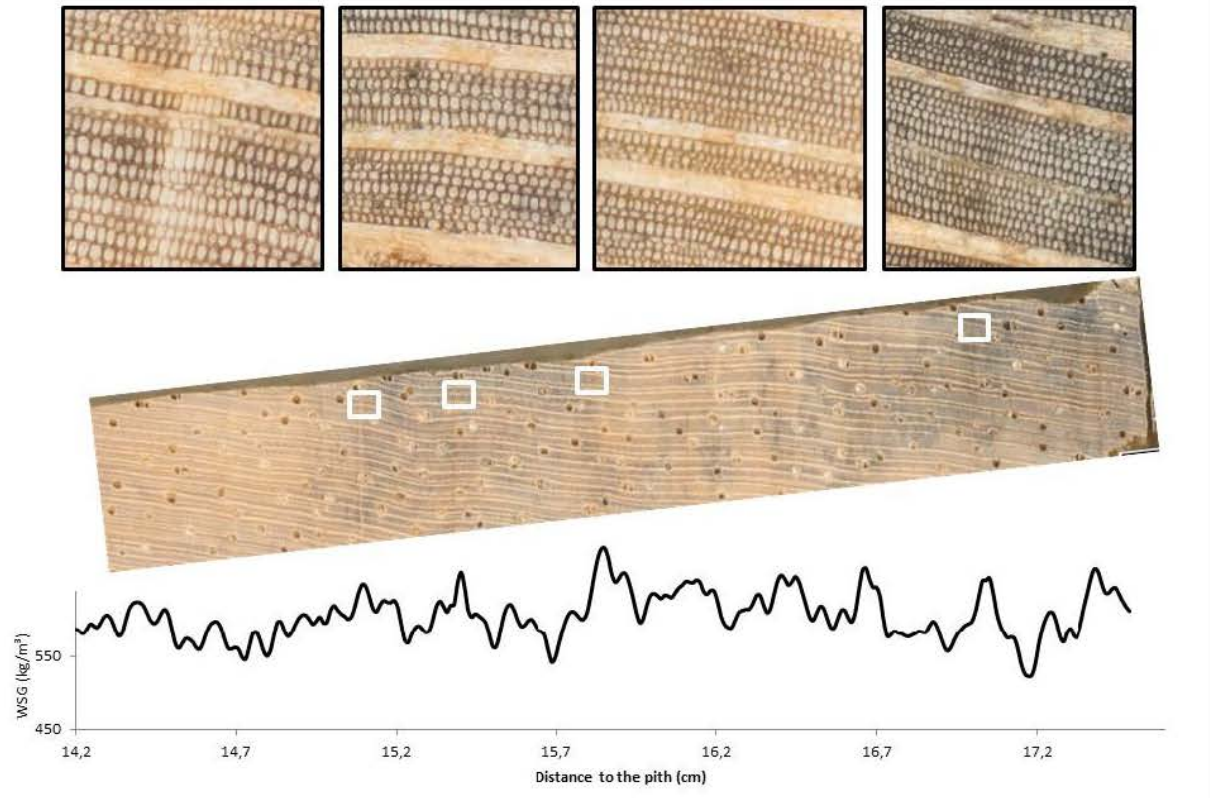


Annex 3

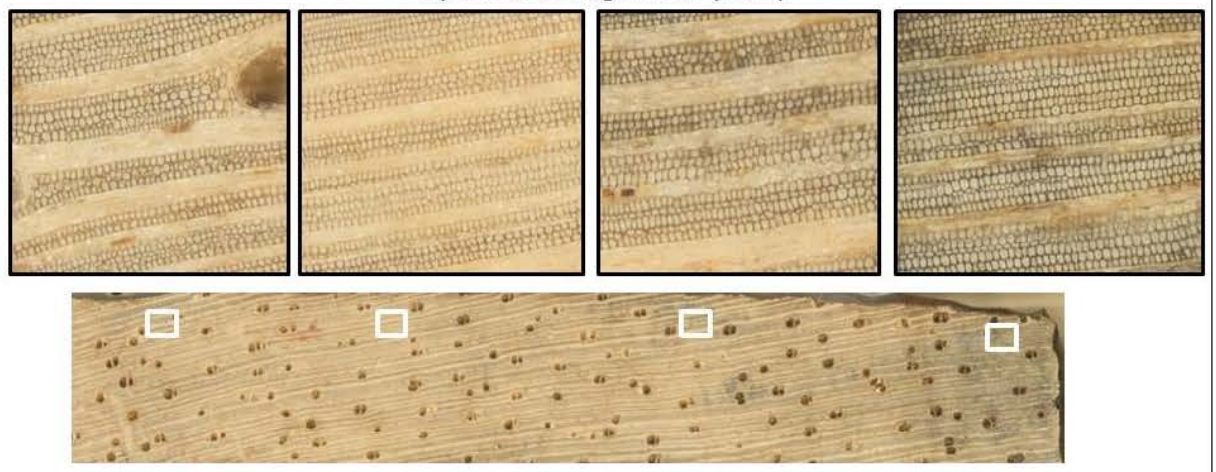
Pycnanthus angolensis (Luki)

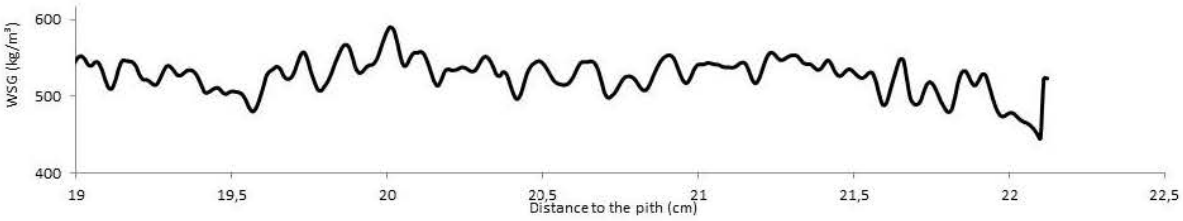


Pycnanthus angolensis (Malebo)

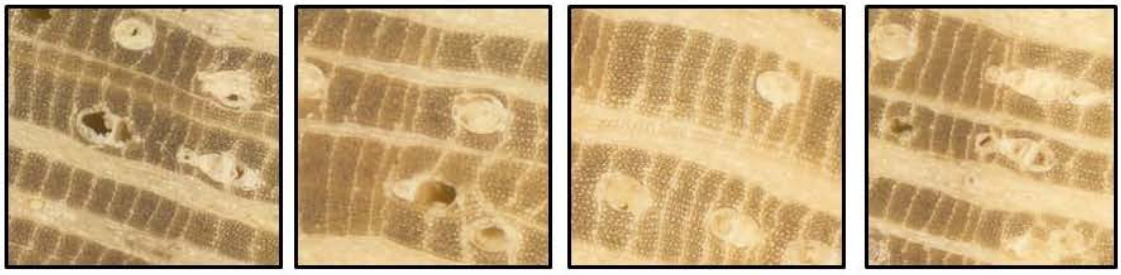


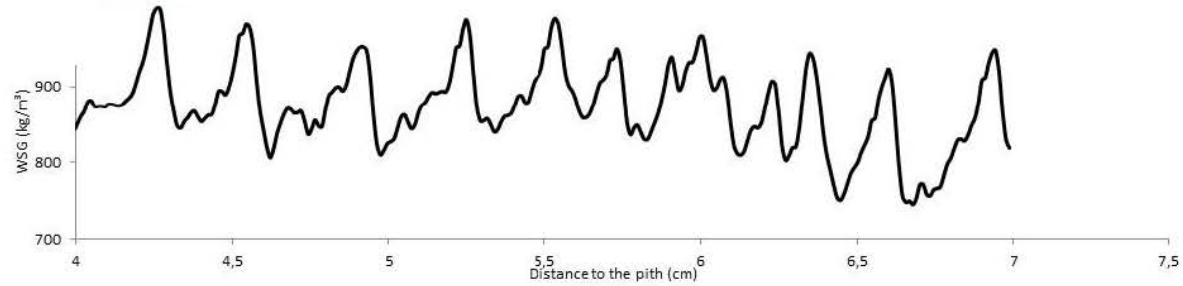
Pycnanthus angolensis (Yoko)





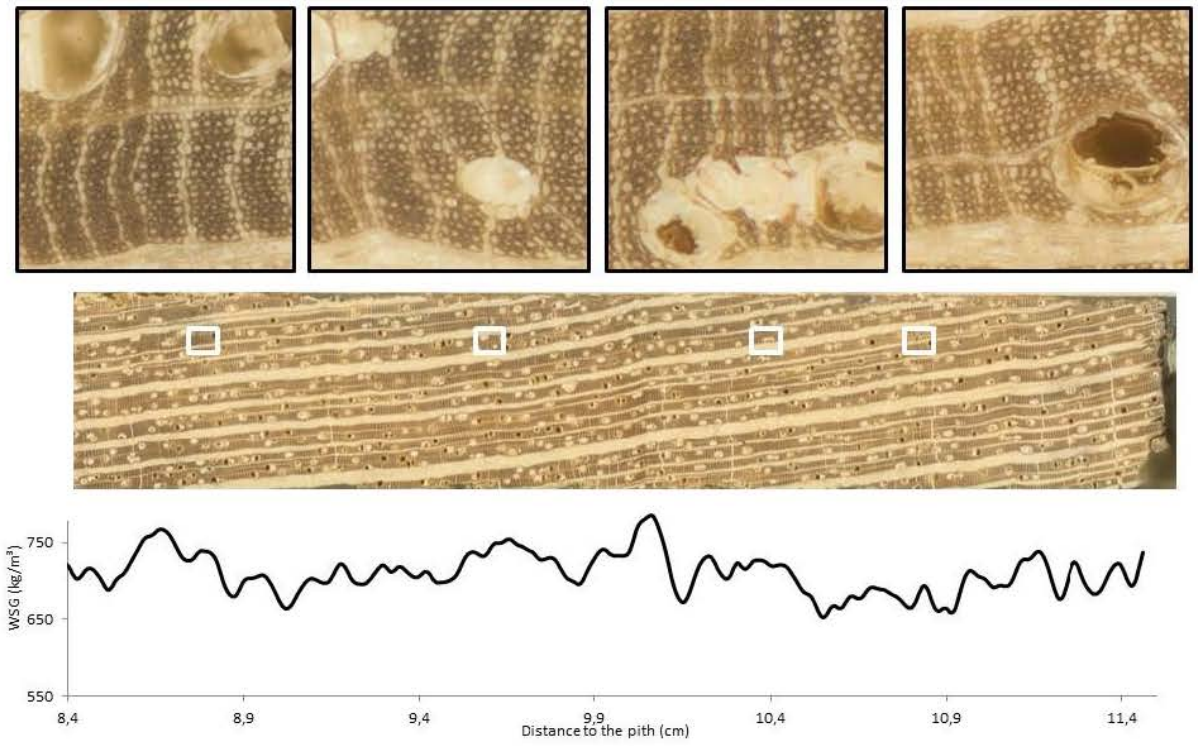
Polyalthia suaveolens (Luki)



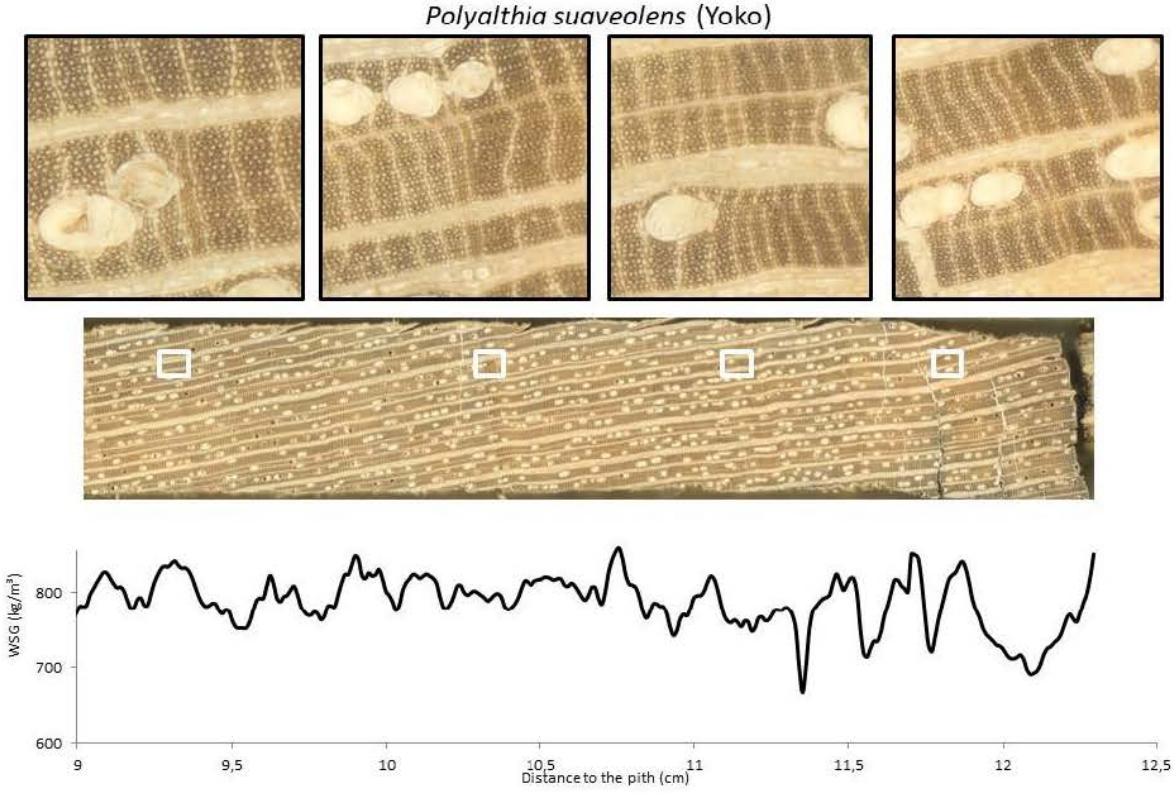


Annex 3

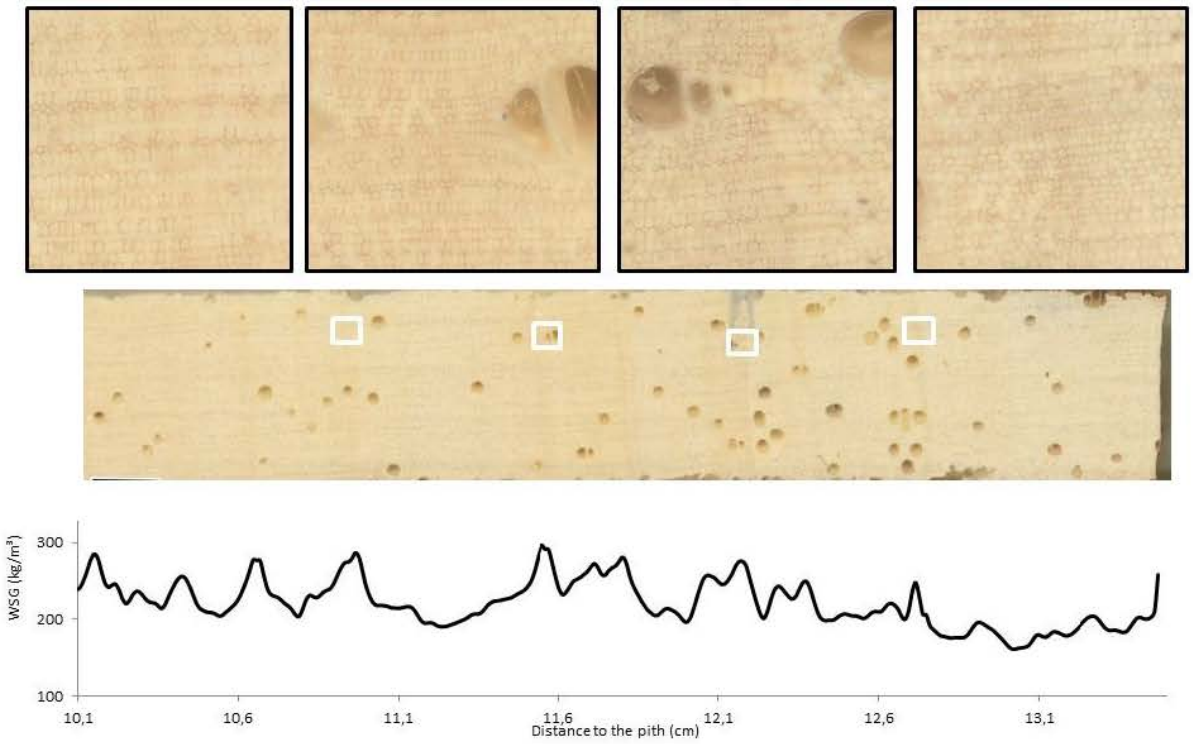
Polyalthia suaveolens (Malebo)



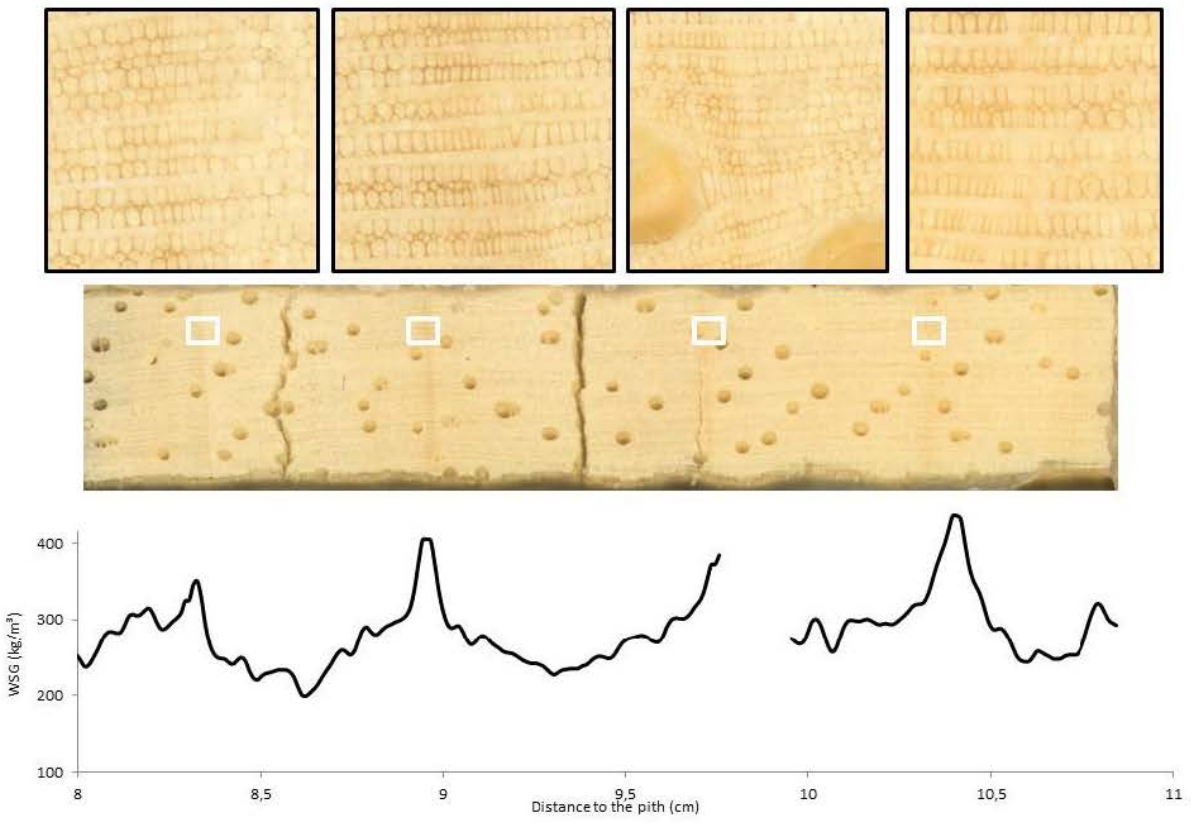
Polyalthia suaveolens (Yoko)



Ricinodendron heudelotii (Luki)

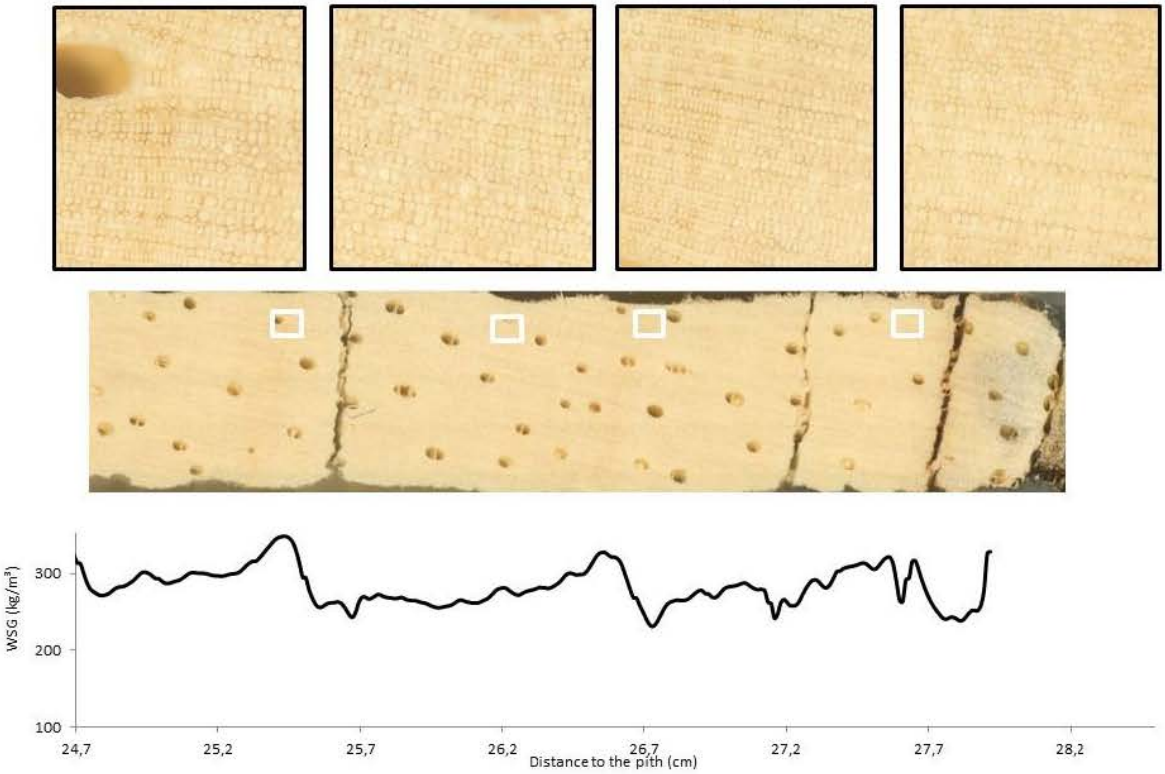


Ricinodendron heudelotii (Malebo)

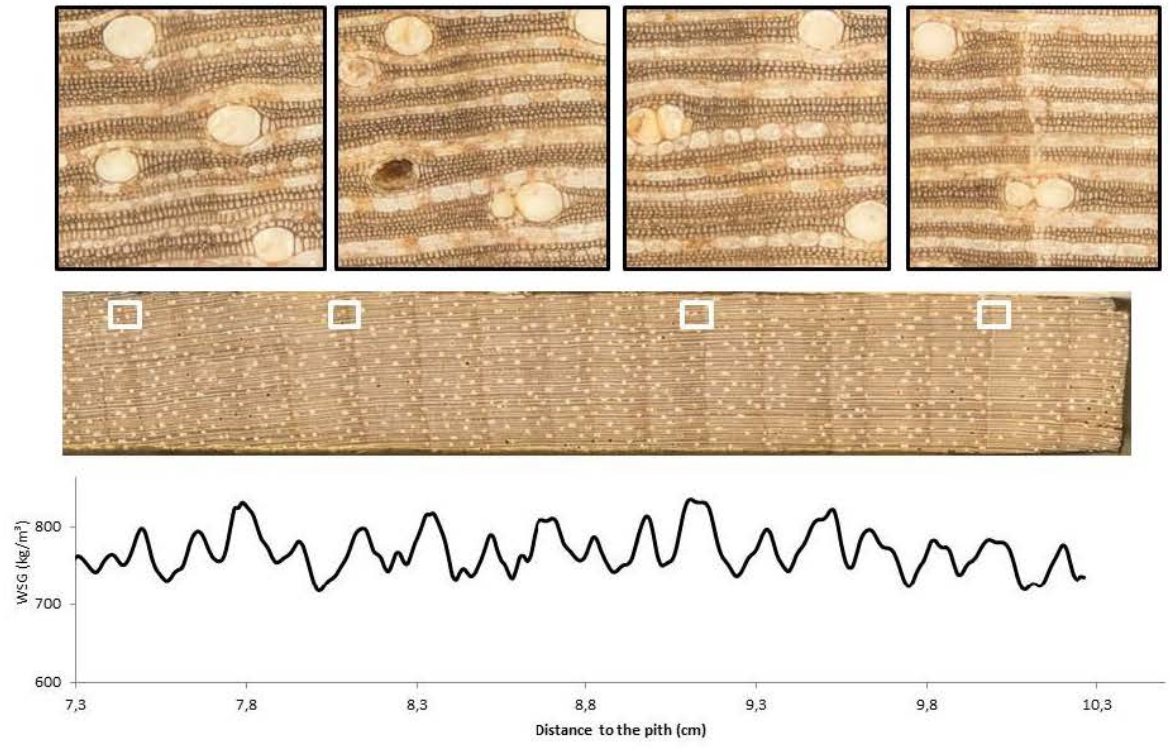


Annex 3

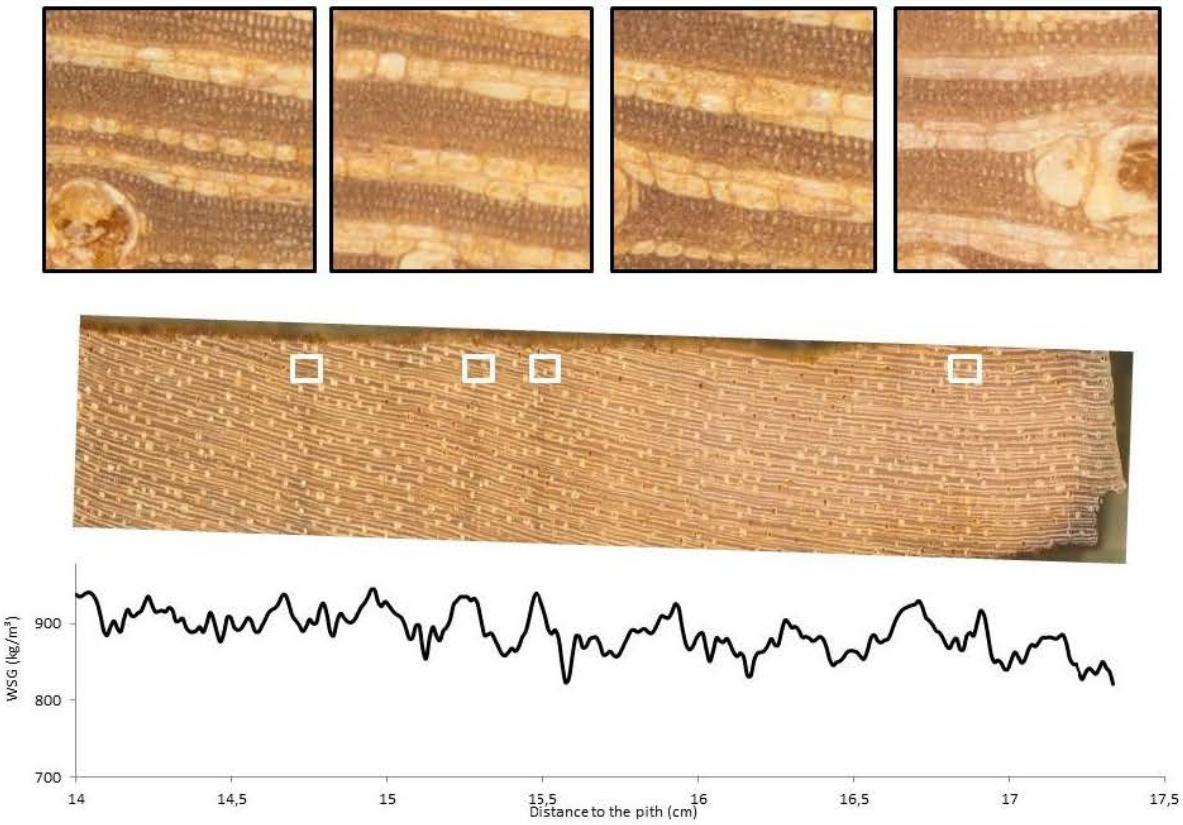
Ricinodendron heudelotii (Yoko)



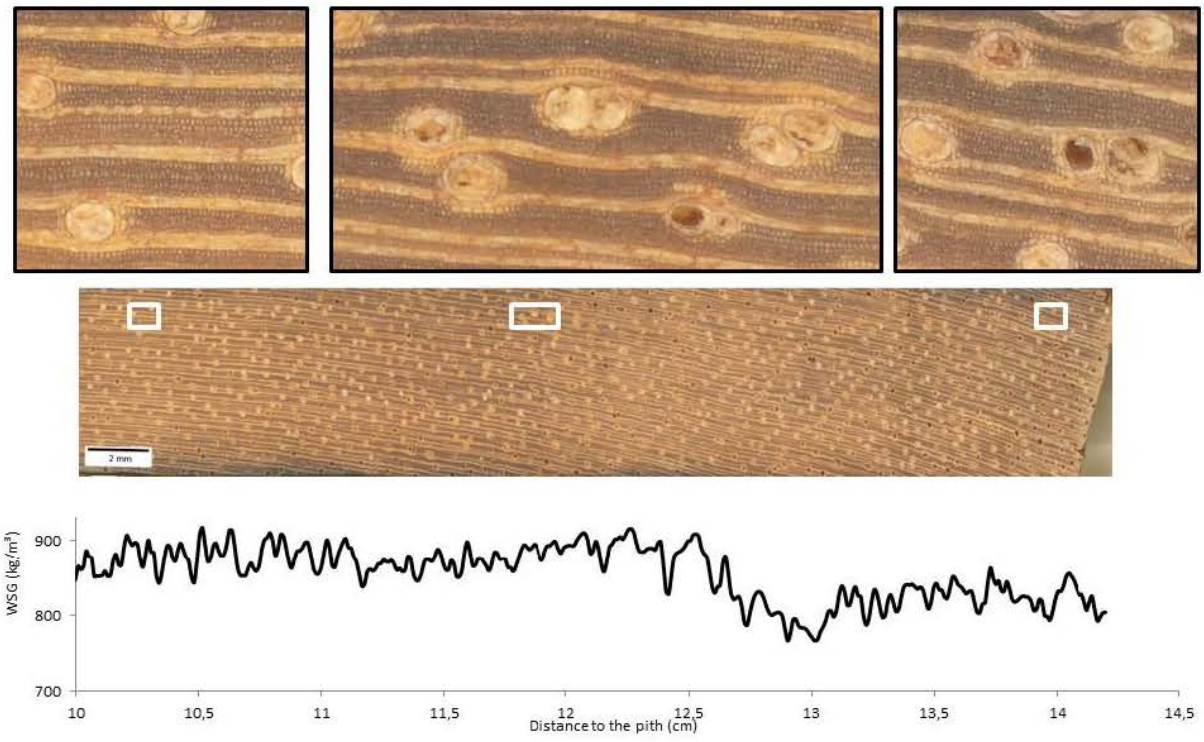
Staudtia kamerunensis (Luki)



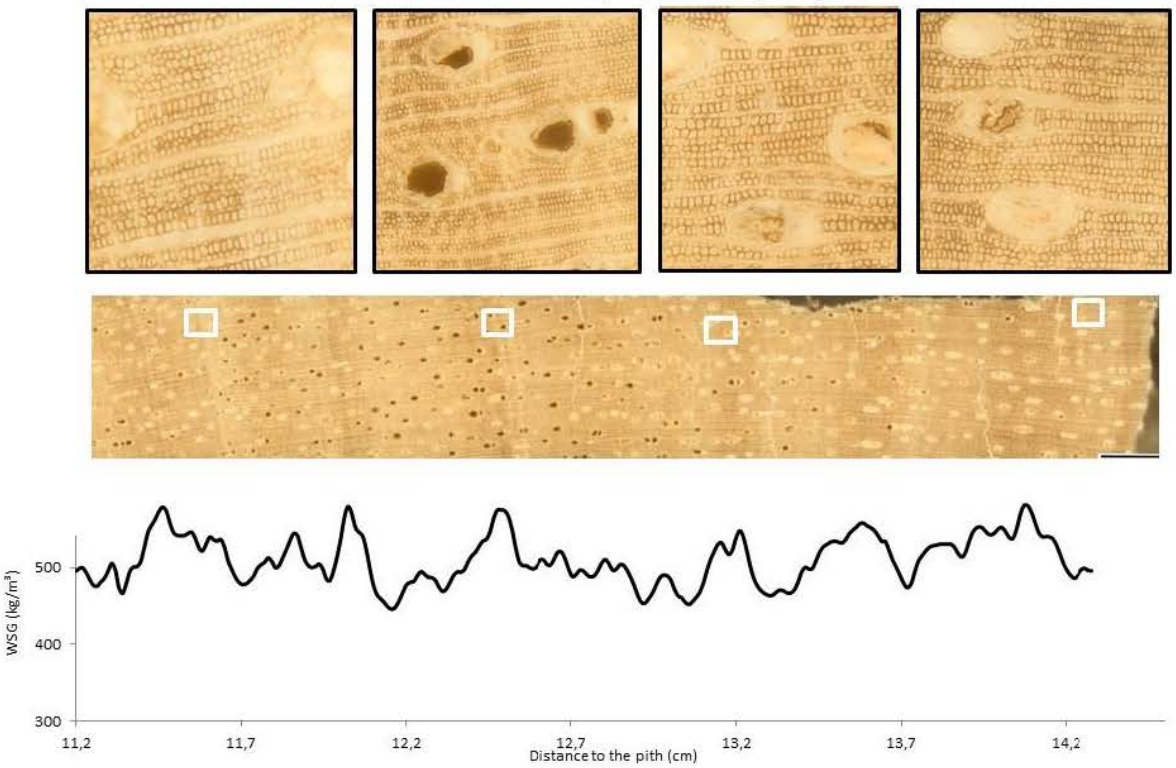
Staudtia kamerunensis (Malebo)



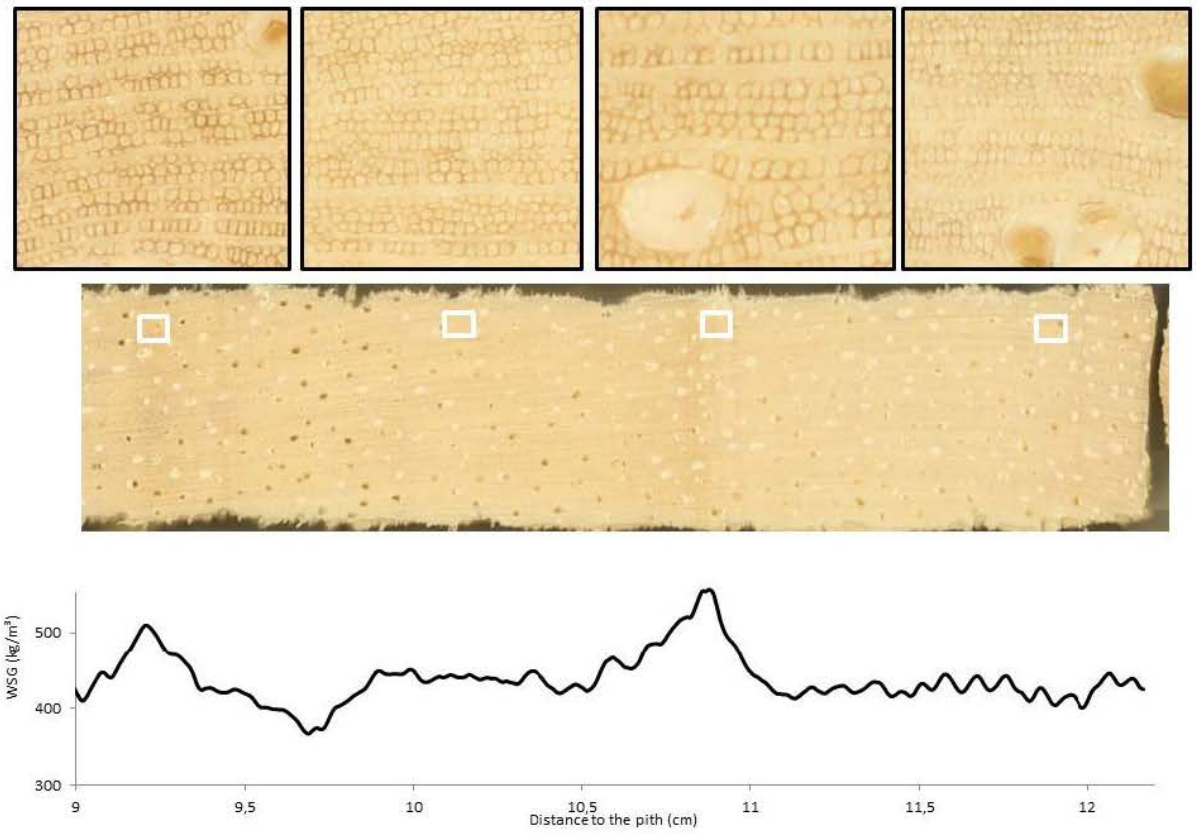
Staudtia kamerunensis (Yoko)

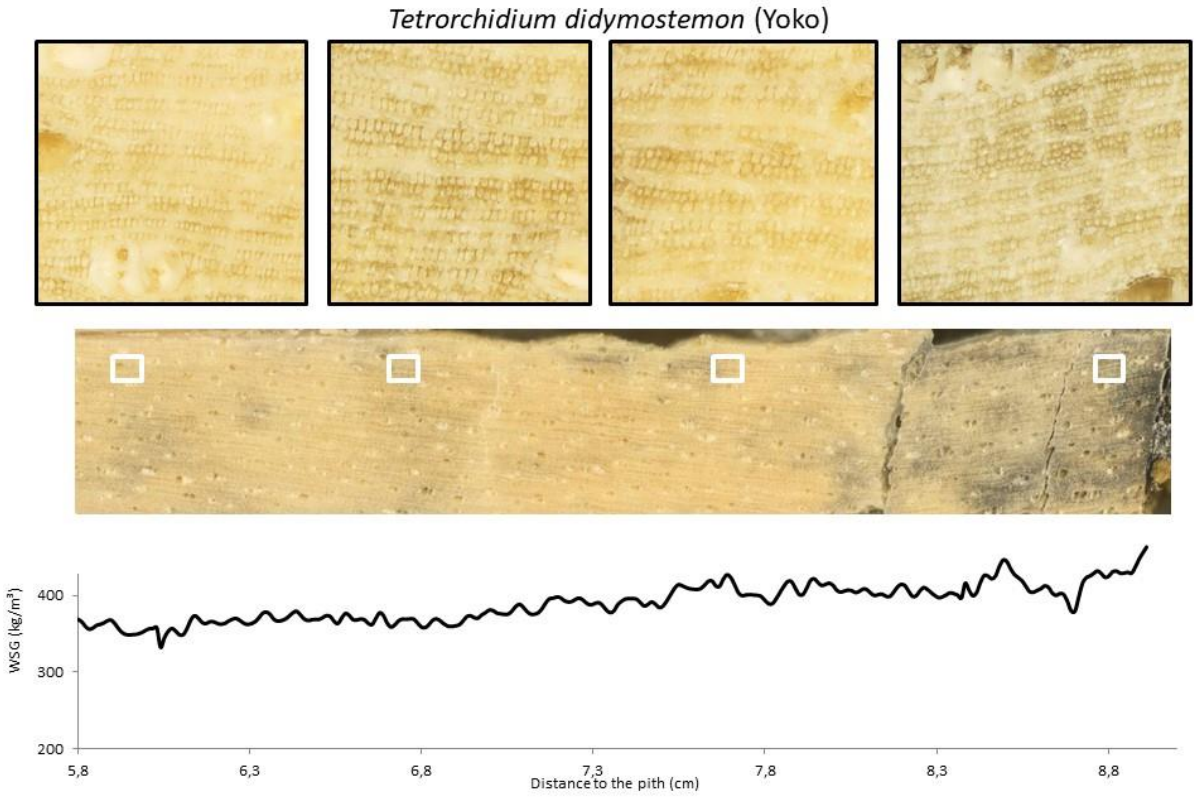


Tetrorchidium didymostemon (Luki)



Tetrorchidium didymostemon (Malebo)





Annex 3:: Supplemental illustrations of the relationship between wood density and wood anatomy. Representation of the last 3 cm under bark of transversal surfaces with four portions zoomed in.

Annex 4: Scientific Curriculum Vitae

Annex 3

Personal

Place Martin Luther King n5 (R4)
1070 Anderlecht
Belgium

Date of Birth: 24/10/1989
Place of Birth: Petropavlovsk, Kazakhstan
Tel: +32 (0) 484 98 77 82
E-mail: yegortarelkin@gmail.com
Driver License: B

Education

- 2013 to 2018: ***PhD student in Applied Biological Sciences: Forest and Nature Management*** at the Landscape Ecology and Plant Production Unit (Université Libre de Bruxelles), Laboratory of Wood Technology (Ghent University) and the Royal Museum of Central Africa.
  PhD dissertation: Radial Patterns of Wood Features Reveal Site-specific Tree Growth Dynamics in the Congo Basin.

- 2011 to 2013: ***MSc Agronomy and Tropical Systems Management*** (Interfaculary School of Bioengineers) at ULB, Belgium. Degree obtained with distinction.
  MSc dissertation: Comparative study of wood density profiles of woody species with contrasted guilds from dense forests of the western DRC.

- 2008 to 2011: ***BSc Bioengineering*** (Interfaculary School of Bioengineers) at ULB, Belgium. Degree obtained with distinction.

- 2002 to 2008: High school, Collège Saint Pierre Uccle, Belgium
  Major in Science and Mathematics

Work experience

- 4 years as a PhD student at the Landscape Ecology and Plant Production Unit (Université Libre de Bruxelles), the Laboratory of Wood Technology (Ghent University) and the Royal Museum of Central Africa, conducting experiments, wood microscopic analysis and data analysis

Experience abroad

- A total of 5 months in the Luki Biosphere forest reserve, Yoko forest reserve and Malebo forests of the Democratic Republic of the Congo (2014 and 2015): leading a team for measuring trees and collecting tree cores for analysis.
- Various stays in Czech Republic and France for international symposiums and training courses

Language

	Reading	Speaking	Writing
French (mother tongue)	5	5	5
Russian (mother tongue)	5	5	5
English	5	4	5
Dutch	3	3	3
Spanish	Notions		

Software

MS Word, Excel, Powerpoint, GIS (Qgis, Grass), tree ring analysis (TSAPWin), Statistics (R), Image processing software (ImageJ, Photoshop, LightRoom), Basic knowledge of MatLab, Notions of C++

Network

Université Libre de Bruxelles (Brussels, Belgium), Ghent University (Ghent, Belgium), Royal Museum of Central Africa (Tervuren, Belgium), Institut de l'étude et recherche agronomiques (Kinshasa, DR Congo), WWF (DR Congo).

Grants

- FRIA (FNRS)
- Fonds Van Buuren et Jaumotte Dumoulin (Special Research Fund ULB)

A1 publications

Published

2016

- Tarelkin, Y., Delvaux, C., De Ridder, M., El Berkani, T., De Cannière, C., & Beeckman, H. (2016). Growth-ring distinctness and boundary anatomy variability in tropical trees. IAWA Journal, 37(2), 275-S7.

2015

- Bastin, J. F., Fayolle, A., Tarelkin, Y., Van den Bulcke, J., De Haulleville, T., Mortier, F., ... & De Cannière, C. (2015). Wood specific gravity variations and biomass of central african tree species: the simple choice of the outer wood. PloS one, 10(11), e0142146.

Annex 3

Conference presentations

2017

- Tarelkin Y, Hufkens K, Hahn S, Debeir O, Bastin JF, Van den Bulcke J, Van Acker J, Beeckman H, De Cannière C. 2017. Phenology and wood anatomical variability. Biospheretraits kickoff meeting, Brussels, Belgium, April 2017

2015

- Tarelkin Y, Delvaux C, De Ridder M, El Berkani T, De Cannière C, Beeckman H. 2015. Tree-ring distinctness: applications in rainforest ecology. COST-STReESS meeting, Praha, Czech Republic, April 2015

Poster presentations

- Tarelkin Y, Hahn S, Bastin JF, Van Den Bulcke J, Debeir O, Van Acker J, Beeckman H, De Cannière C. 2015. Pith-to-bark variation in wood density and anatomical features as a potential tool for dendrochronology of tropical tree species. WSUFEM (Wood science underpinning tropical forest ecology and management) conference, Tervuren, Belgium, May 2015

Supervision of Msc students:

- Thomas El Berkani (2015-2016). Étude dendrochronologique des variations de l'activité cambiale chez Entandrophragma angolense et Ricinodendron heudelotii

- Manoe De Neck (2015 - 2016). Interprétation anatomique de profils de densité du bois de Staudtia kamerunensis provenant des forêts du bassin du Congo